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Development and Evaluation of Analytical Test Procedures for Priority Pollutants

Gulf South Research Inst.
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DEVELOPMENT AND EVALUATION OF ANALYTICAL TEST PROCEDURES FOR PRIORITY POLLUTANTS

by

C. S. Monteith
Gulf South Research Institute
New Orleans, Louisiana 70186

Contract No. 68-03-2779

Project Officer
D. F. Bender
Environmental Monitoring and Support Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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16. ABSTRACT

Analytical methods were developed for the determination of cyanide and of total phenolic compounds in solid/semi-solid samples of environmental importance. A number of methods were reviewed, selected methods were empirically evaluated, and the most promising methods were optimized and validated by application to ten solid/semi-solid matrices. Each method consisted of two steps: isolation of the analyte and quantification of the analyte. Isolation studies were performed using radiolabeled cyanide and radiolabeled phenolic compounds to spike representative matrices.

The method which was selected for the determination of cyanide in solid/semi-solid matrices involves distillation of cyanide from, an acidified slurry of the sample, followed by quantification using the pyridine-barbituric acid procedure. The method which was selected for the determination of total phenolic compounds involves extraction of an acidified slurry of the sample with methylene chloride in a blender. The methylene chloride is then extracted with a sodium hydroxide solution and quantified using the 4-aminoantipyrene (4-AAP) procedure.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report describes the evaluation of methods for the analysis of cyanide compounds and phenolic compounds in ten solid and semisolid matrices which are of environmental importance. The methods were optimized primarily on bottom sediments. These data, and the conclusions which can be drawn from them, will be an important part of future decisions and future recommedations concerning methods of analysis for solids and semisolid samples which are related to the pollution of our water sources.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support
Laboratory - Cincinnati

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ABSTRACT

The goals of this research program were to develop analytical methods for cyanide and total phenolic compounds in solid and semisolid environmental samples. The program was divided into four phases: review of the literature and selection of methods, empirical evaluation of methods, optimization of promising methods, and validation of the optimized methods. Procedures for isolation and quantification were evaluated independently. Isolation procedures were evaluated using solid and semisolid environmental samples spiked with radiolabeled cyanide or phenolic compounds. Quantification procedures were tested initially on aqueous solutions of cyanide or phenol. Promising isolation and quantification procedures for cyanide or phenolic compounds were united to form a single method for analysis of solid and semisolid samples. These methods were optimized and validated by analysis of samples of ten solid and semisolid environmental matrices. Preservation procedures for these samples were also investigated.

The results of this research effort are methods for analysis of solid and semisolid environmental samples for cyanide and total phenolic compounds. The method for determination of cyanide is distillation of cyanide from an acidified slurry of the sample followed by quantification using the pyridine-barbituric acid procedure. Phenolic compounds are extracted from acidified slurries of solid and semisolid samples by blending with methylene chloride. The phenolic compounds are extracted from the methylene chloride into sodium hydroxide solution and quantified using 4-aminoantipyrine solution.

This report was submitted in fulfillment of Contract No. 68-03-2779 by Gulf South Research Institute under the sponsorship of the U. S. Environmental Protection Agency, and covers a period from February 12, 1979, to February 11, 1981, and work was completed as of February 11, 1981.

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SECTION 1

INTRODUCTION

Cyanide and phenolic compounds are common pollutants of water bodies and land disposal sites. Cyanide complexes are found in the wastewaters of many industrial processes, including electroplating, precious metal refining, and steel hardening. They are used as intermediates in polymer synthesis and fumigants in produce storage areas. Wastes containing phenolic compounds are generated by the petroleum, plastics, and organic chemical industries. Cyanide and phenolic compounds contained in wastewaters discharged into natural water bodies may accummulate in the sediments or be taken up by aquatic plants and animals. Materials containing cyanide or phenolic compounds such as solid wastes from industrial and domestic sources, slags from mining and industrial processing, and fly ash from commercial operations and power generation are deposited in landfill sites. As a result, it is desirable to study the transport, fate, and toxicity of cyanide and phenolic compounds in the environment.

The goals of this research program were to develop methods for analyzing solid and semisolid environmental samples for cyanide and phenolic compounds. Preservation procedures for these samples were also investigated to insure that the concentrations of cyanide and phenolic compounds determined in the laboratory are accurate measurements of levels present in the environment. The program consisted of four phases:

Phase I:	Selection of methodology from the literature and experience of Gulf South Research Institue and the U. S. Environmental Protection Agency (U.S. EPA).
Phase II:	Experimentation to evaluate the methods, eliminate nonviable alternatives, modify and optimize procedures, and identify necessary developmental work.
Phase III:	Experimentation to optimize the methods, application of the methods to real world samples to identify potential problems, and determination of accuracy and precision of the methods.
Phase IV:	Statistical validation of the methods optimized in Phase III.

The literature search performed in Phase I identified the gaps in knowledge associated with preservation and analysis of solid and semisolid environmental samples containing cyanide and phenolic compounds. The information gathered was used to develop a plan for preliminary experiments to establish viable preservation and analysis procedures.

Separation and quantification procedures were evaluated independently for application to solid and semisolid environmental samples. The results of these experiments were used to identify an analytical method for cyanide and one for phenolic compounds. During Phase III these techniques were optimized by application to solid and semisolid environmental samples. The methods were validated in Phase IV by the analysis of samples of ten solid and semisolid environmental matrices. Duplicate analyses were performed on spiked and unspiked samples of each matrix. The accuracy, precision, and sensitivity of the methods are described.

This research effort has produced methods for the analysis of solid and semisolid environmental samples for cyanide and total phenolic compounds. The recommended method for determination of cyanide is distillation from an acidified slurry of the sample followed by quantification using the pyridine-barbituric acid procedure. Phenolic compounds are extracted from solid and semisolid samples by blending with methylene chloride. The phenolic compounds are subsequently extracted from the methylene chloride into a sodium hydroxide solution and quantified using 4-aminoantipyrine solution.

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SECTION 2

RECOMMENDATIONS AND CONCLUSIONS

A review of the literature has identified the lack of information on analysis of solid and semisolid environmental samples for cyanide and total phenolic compounds. The methods which were developed in this program are adaptations of methods for determination of cyanide and phenolic compounds in aqueous samples. The method for analysis of solid and semisolid environmental samples for cyanide is distillation of hydrogen cyanide from an acidified slurry of the sample. The distillate is bubbled through a solution of lead acetate to remove sulfur-containing compounds which would interfere in the determination procedure. The hydrogen cyanide is collected in sodium hydroxide solution and quantified using pyridine-barbituric acid solution. Levels of cyanide in a sample as low as 5 μg can be detected by the method. This translates to a detection limit of 0.5 μg/g for analysis of a 10-g sample. The method was validated by analysis of sixty spiked and unspiked solid and semisolid environmental samples of ten matrices. Recovery of cyanide at concentrations of 2.6 to 20 μg/g from these samples ranged from 69 to 112 percent, with an average of 90 ± 10 percent.

The method of analysis of solid and semisolid environmental samples for total phenolic compounds is also an adaptation of methods in the literature for analysis of aqueous samples. Phenolic compounds are extracted from solid and semisolid samples by blending with methylene chloride. The phenolic compounds are subsequently extracted from the methylene chloride into sodium hydroxide solution and quantified by the 4-aminoantipyrine procedure. The method was validated by analysis of sixty solid and semisolid environmental samples spiked with phenol. Recovery of phenol ranged from 22 to 100 percent with an average of 50 ± 20 percent. Recovery appears to be dependent on sample matrix, with higher recoveries being obtained from inorganic matrices such as sediment, fly ash, and industrial processing slag. The method is sensitive to approximately 1 μg of phenol. Phenol concentrations as low as 0.04 μg/g can be determined in a 25-g sample.

A round-robin study should be performed to validate the methods developed in this program. A large number of solid and semisolid environmental samples should be analyzed for cyanide and total phenolic compounds.

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SECTION 3

METHOD FOR ANALYSIS OF SOLID AND SEMISOLID ENVIRONMENTAL SAMPLES FOR CYANIDE

INTRODUCTION

An extensive search of the literature was performed to find methods for preservation and analysis of samples of all matrices containing cyanide. Numerous methods for quantification of cyanide in aqueous samples have been published, but limited information is available on analysis of solid or semisolid matrices. Methods which appeared to be appropriate for application to the analysis of solid and semisolid environmental samples were selected for empirical evaluation. Selection criteria used in evaluating the methods in the literature were accuracy and analytical range of the method, lack of significant interferences, minimal sensitivity to changes in sample pH or temperature, and ease of performing the determination.

Method evaluation was approached as a two-step procedure. The cyanide was separated from the matrix and then quantified. Two separation procedures, distillation and solvent extraction, were evaluated. Solid matrices were spiked with radiolabeled cyanide and subjected to the separation procedure. Liquid scintillation counting was used to determine recovery of the radiolabeled cyanide, thus avoiding reliance on an untested quantification method for cyanide in solid samples. The procedures evaluated for quantification of cyanide included five colorimetric procedures, a cyanide ion-selective electrode, and electron capture gas chromatography. These procedures were performed on aqueous solutions containing a known concentration of cyanide.

In the optimization phase, distillation and the pyridine-barbituric acid colorimetric procedure were joined to form a unified method for determination of cyanide concentrations in solid and semisolid environmental samples. A review of the literature revealed that the most significant interference to this determination method is the positive response of volatile sulfur-containing compounds. These materials were removed by bubbling the distillate through lead acetate solution. Removal of interferences and recovery of cyanide were monitored using the pyridine-barbituric acid colorimetric procedure and the cyanide ion-selective electrode. Comparison of the results of these two determination procedures, which respond differently to the presence of sulfur-containing compounds, allowed the experimental conditions to be optimized to minimize interferences.

The precision, accuracy, and sensitivity of the method were determined. Replicate samples of solid environmental matrices were analyzed for cyanide.

These matrices were spiked with three levels of cyanide and analyzed. The percentage of cyanide recovered and the standard deviation of the replicates were calculated. The method was validated by analysis of duplicate samples of ten different matrices. These matrices were spiked at two concentrations with cyanide and analyzed in duplicate.

PHASE I: SELECTION OF METHODOLOGY FROM THE LITERATURE

A search of the literature for information on preservation and analysis of solid and semisolid environmental samples containing cyanide was conducted in Phase I. Eighty-nine papers concerning analysis for cyanide were retrieved using computerized and manual literature searching techniques. While some information has been published on determination of cyanide in biological matrices, the majority of methods reported in the literature are for analysis of aqueous samples. The subject of preservation of solid and semisolid environmental samples containing cyanide has not been adequately addressed.

The information retrieved from the literature was classified by type of isolation or quantification procedure. The isolation procedures discussed are distillation and microdiffusion. Quantification procedures include a variety of colorimetric and fluorometric analyses, titrations, ion-selective electrodes, gas chromatography, and miscellaneous instrumental techniques. A review of the literature is presented below.

Methods of Isolation

The term cyanide refers to all CN groups in compounds which can be determined as the cyanide ion, CN^-. There are numerous salts of cyanide, known as simple cyanides, which can be represented by the general formula $A(CN)_x$ where A represents an alkali or metal and x equals the valence of A and is the number of CN groups. Cyanide salts of silver (I), mercury (I), and lead (II) are insoluble, whereas mercuric cyanide, $Hg(CN)_2$, is moderately soluble in water. Cyanide metal complexes are restricted almost entirely to the transition metals of the d block and their near neighbors zinc, cadmium, and mercury. The majority of cyano complexes have the general formula

$$[M^{n+}(CN)_x]^{(x-n)-},\ A_y(CN)_x,\ \text{or}\ [M(CN)_5Z]^{n-}$$

where M is a metal, A is an alkali, and Z is H_2O, NH_3, CO, NO, H, or a halogen. The dissociation constants of the complexes vary greatly. The more refractory complexes of metals such as nickel, cobalt, and iron, referred to as complex cyanides, require rigorous treatment to free the cyanide ion for determination.

Isolation of cyanide from a matrix is generally based on the fact that hydrogen cyanide is more volatile than other components of the sample. Separation of total cyanide from a sample requires that all cyanide complexes be converted to the volatile form, hydrogen cyanide. Generally, only acidic conditions are needed to degrade simple cyanides. Complex cyanides can be broken down by heating in the presence of a catalyst or irradiating with ultraviolet (UV) light. The addition of a catalyst and application of heat are easily coupled with distillation and will be discussed later. UV irradiation has been used to decompose complex cyanides in water prior to chromatographic analysis (1) or sample distillation (2-4).

Distillation--

The basic methods of isolating cyanide from a sample matrix are distillation and microdiffusion. The application and rigor of these methods vary. Distillation, commonly used to separate cyanide from aqueous samples, has the advantage of being able to break down most complex cyanide compounds under proper conditions. Sample pH and the presence of a catalyst are the factors controlling the generation of hydrogen cyanide from cyanide compounds. A stream of air or nitrogen is passed through the distillation apparatus to transfer hydrogen cyanide from the sample to an absorbing solution such as sodium hydroxide. Sulfuric acid is commonly used to adjust the pH of the sample (4-9). Tartaric acid (7,10), hydrochloric acid (11), hypophosphoric acid (3), phosphoric mercaptoacetic acid (12), and sodium bicarbonate (11) have also been used to lower sample pH. The recovery of simple and complex cyanide from body fluids by distilling the sample in the presence of hydrochloric acid, sodium bicarbonate, and pH 6.5 buffer salt was studied (11). Recovery of cyanide was found to increase with a decrease in pH.

Catalysts such as magnesium chloride (7,13-15), mercuric chloride (7,14,15), and cuprous chloride (9,13,16) have been added to the sample to increase the recovery of cyanide from refractory compounds. The recovery of simple and complex cyanides from aqueous samples distilled in the presence of sulfuric acid and the catalysts magnesium chloride and mercuric chloride (Serfass distillation), and tartaric acid with no catalysts was studied (7). All simple cyanides and complex cyanides of zinc, cadmium, nickel, and silver are easily distilled by either procedure. Ferro- and ferricyanides can also be recovered quantitatively by either method. Recovery of complex copper cyanides was high only with Serfass distillation, while recovery of cobalticyanides was only 20 percent with this method and very low with tartaric acid distillation (7). Formation of mercuric cyanide which is not recovered by distillation if the chloride concentration is insufficient can be prevented by using a higher ratio of magnesium chloride to mercuric chloride (14). Over 99 percent of the ferrocyanide in water can be recovered by distillation in the presence of cuprous chloride and sulfuric and hydrochloric acids (16). The method recommended by the American Public Health Association (APHA) (13) calls for addition of sulfuric acid and cuprous chloride or magnesium chloride to the sample prior to distillation, while the method recommended by the U.S. EPA (9) utilizes magnesium chloride and sulfuric acid.

The apparatus used and length of time allowed for distillation of cyanide vary. The most commonly used apparatus (3,7,9,11,13,14) for fractional distillation was devised by Serfass (15) which consists of a distillation flask equipped with an Allihn condenser attached to a gas washer and a vacuum supply (Figure 1). Steam distillation has been used to separate cyanide from blood and tissue (17). Simple cyanide can be separated from ferrocyanide by distillation under reduced pressure (18). The distillation process has been automated (2), and a microstill has been used for isolating hydrogen cyanide from insects (10).

Aeration has been used as a method for separation of hydrogen cyanide from biological (19-24) and water samples (25). The sample is acidified, and held at room temperature or warmed. A stream of air or nitrogen is passed through or over the sample to sweep the hydrogen cyanide into an absorbing solution or onto a packed column. The major factors affecting the efficiency of isolating hydrogen cyanide from samples by aeration have been found to be sample pH and temperature (21).

Microdiffusion--

A frequently used method of isolating cyanide from biological samples is microdiffusion (26-32). A sample of blood, urine, gastric contents, or homogenized tissue and an aliquot of sulfuric acid are placed in the outer chamber of a Conway microdiffusion cell. Sodium hydroxide solution is placed in the inner chamber, and the cyanide is allowed to diffuse from the sample into the sodium hydroxide solution. The Cavett blood-alcohol flask has been modified for use as a diffusion cell (31). The microdiffusion technique isolates only volatile components from the sample; therefore, it can not be used to determine total cyanide without sample pretreatment. The volume of sample which can be contained in the microdiffusion cell is small (less than 5 ml); thus, the detectable concentration in the sample is severely limited by sample size.

Methods of Quantification

The majority of methods for quantification of cyanide are based on the reaction of cyanide ions with a colorimetric reagent. In general, these spectrophotometric methods are accurate and sensititive. Fluorometric methods have been developed which have sensitivities comparable or superior to spectrophotometric methods, but they require more sophisticated instrumentation. Titration methods, while accurate, are not sensitive enough to determine traces of cyanide in environmental samples. Satisfactory instrumental methods using ion-selective electrodes, gas chromatography, coulometry, and atomic absorption spectrophotometry have been developed in recent years.

Spectrophotometric Methods--

Konig reaction--A basic method for the determination of cyanide in aqueous (33-36) and biological samples (11,23,26) is based on a Konig reaction (37). A common method (37) utilizes Konig synthesis of a pyridine dye in which cyanogen bromide or cyanogen chloride reacts with pyridine and an aromatic amine to form a dye. The cyanide is brominated, excess bromine is

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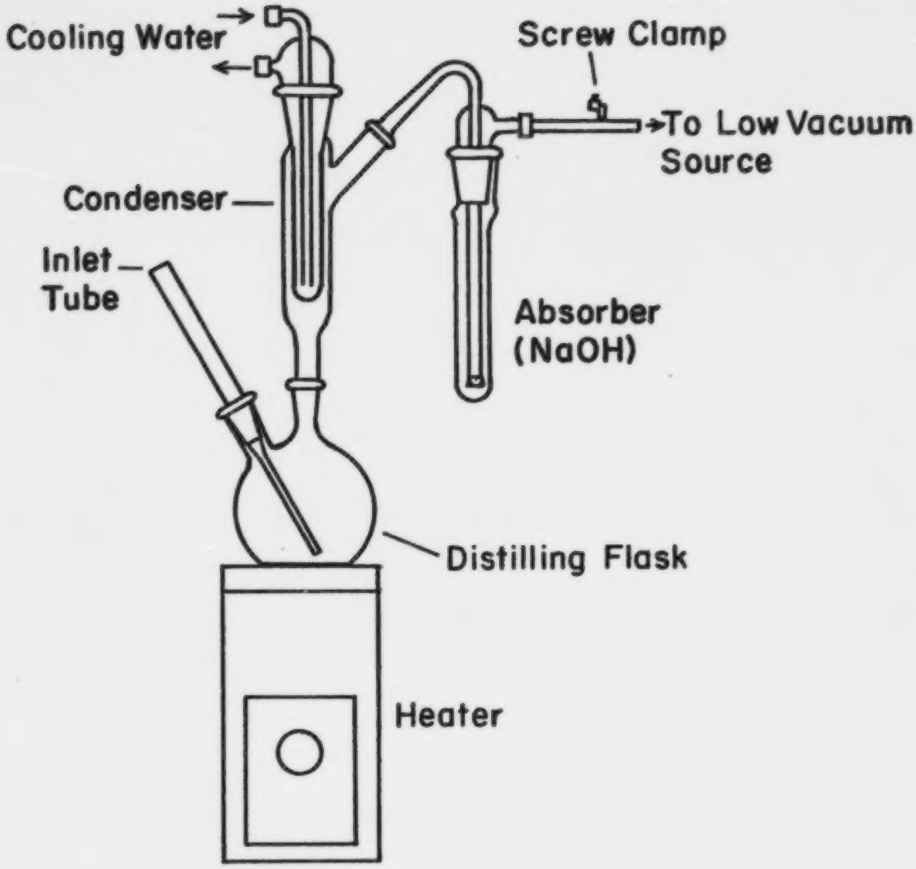
Cooling Water
Screw Clamp
To Low Vacuum Source
Condenser
Inlet Tube
Absorber (NaOH)
Distilling Flask
Heater

Figure 1. Apparatus for distillation of cyanide.

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removed with arsenious acid, and the cyanogen bromide reacts with a mixed pyridine-benzidine reagent. The absorbance of the dye is proportional to the concentration of cyanide in the sample. Concentrations of cyanide as low as 0.1 mg/L can be determined with an error of ± 2 percent (38). This method has been used to determine cyanide in blood, urine, and pancreatic juices deproteinized with trichloroacetic acid (22,38) or distilled with acid (11). A comparison of seven amines with pyridine and forty-six aromatic amines with benzidine was made in a search for noncarcinogenic reagents which are more stable and have a greater molecular extinction coefficient than pyridine and benzidine (33,34). By substituting p-phenylenediamine for benzidine and slightly altering the procedure, concentrations of cyanide ranging from 0.005-100 mg/L can be determined (39). In recent years this method has been used for analysis of biological fluids (23,26,40) and adapted for automated analysis of water and wastewater (35,36).

The Konig synthesis using pyridine and benzidine or pyridine and p-phenylenediamine is sensitive to thiosulfate and sulfide as well as cyanide. Interference from thiocyanate, turbidity, and color can be eliminated by distillation of the sample, and sulfide can be removed by precipitation with cadmium. The intensity of the dye is sensitive to the pH and temperature of the test solution and the time allowed for development of the dye. These factors can be controlled easily enough to render the method viable.

The intermediate cyanogen chloride can be replaced with the less volatile cyanogen bromide, and barbituric acid can be used as the condensing agent for the reaction with pyridine (41). The stability of the dye formed using cyanogen bromide with pyridine and barbituric acid is superior to that of the dye formed in the cyanogen chloride-barbituric acid method or the benzidine or pyrazolone methods. Recovery of 96-102 percent at cyanide levels of 0.1-2.0 µg in plasma, urine, and tissue samples using cyanogen chloride with pyridine and barbituric acid has been reported (28). The American Public Health Association (13) and U.S. EPA (9) also advocate the cyanogen chloride-pyridine-barbituric acid method for determination of cyanide in water samples.

A method (42) for determination of cyanide in water is recommended by the U.S. EPA (9) as an alternative to the pyridine-barbituric acid method and has been used by many for the determination of cyanide in wastewater (2,18,43). Chloramine-T is used to convert cyanide to cyanogen chloride. The cyanogen chloride reacts with a solution of pyridine containing 0.1 percent bis-pyrazolone and 1-phenyl-3-methyl-5-pyrazolone to form a dye which is stable for 30 minutes at 25°C. The absorbance of the dye measured at 630 nm is proportional to the concentration of cyanide in the sample. The advantages of this method are that as little as 0.2 µg of cyanide can be determined with an accuracy of 99 ± 4 percent, and that there is no change in intensity of the dye with pH changes in the range 2.8-9.0. High concentrations of reducing agents may interfere with the determination (42). This method has been adapted to the analysis of biological samples including blood, urine, tissue, and gastric contents (3,14,19,21,28,31). The pyridine-pyrazolone dye can be extracted with butyl alcohol to increase the sensitivity of the method (7,44). The color reaction is generally reproducible over a broad range, but a significant buffer capacity of a sample will decrease the effective pH range (7,44).

Metallic reactions--One of the earliest methods for determination of cyanide involving formation of a metal complex was based on the formation of Prussian blue, $Fe_4[Fe(CN)_6]_3$, by the reaction of cyanide with ferrous sulfate (10,45). This method is sensitive (0.01 mg hydrogen cyanide), but has many shortcomings. The age of the ferrous sulfate solution causes the color to vary significantly. The presence of air during the reaction period influences the size of the colloidal particles and thus the photometer readings. Improved methods of analysis based on the reaction of cyanide with 1,10-phenanthroline were reported (46-48). A method based upon formation and extraction of the neutral dicyano-bis(1,10-phenanthroline) iron (II) complex produced by the exchange reaction between tris(1,10-phenanthroline) iron (II) and cyanide ions was developed (46). The color produced is stable for up to an hour. Disadvantages of the method include interference by periodate, cobalt, copper, nickel, and ferrous ions; incomplete recovery of the dicyano-bis(1,10-phenanthroline) iron (II) complex by extraction; and extreme sensitivity to variation of pH (46).

Another procedure is based on the reaction of tris(1,10-phenanthroline) iron (II) triiodide adsorbed to porcelain chips with cyanide to release the red complex cation (47). Absorbance at 514 nm by the red complex is linear up to 8 mg/L. None of several ions added to the sample interfered with the determination.

An indirect spectrophotometric method is based on the fact that cyanide prevents formation of the strongly absorbing ternary complex of silver (I), 1,10-phenanthroline and bromopyrogallol red in nearly neutral aqueous solution (48). A plot of absorbance versus concentration is linear over the range 0.26-2.6 mg/L of cyanide. Several anions and cations interfere with the determination, but most of these can be eliminated by precipitation or masked with EDTA.

The application of mercury chloranilates for the spectrophotometric determination of cyanide was evaluated (49). The reaction of cyanide with mercury chloranilate forms soluble, undissociated mercuric cyanide. Cyanide concentration can be determined over the range of 0.4-4.0 mg/L by measuring the absorbance of the solution at 330 nm. The method is hindered by the significant interference of sulfite and sulfide.

A procedure for quantification of cyanide using ammoniacal nickel chloride was developed (5). Hydrogen cyanide is distilled from the sample and collected in a solution of ammoniacal nickel chloride. The cyanide reacts immediately to form an ionic complex which can be quantified by ultraviolet spectrophotometry. Hydrogen sulfide and sulfite must be removed prior to analysis to prevent interferences. The method follows Beer's law over the range of 0.1 to 4.0 mg/L of cyanide.

Organic disulfide reactions--The reaction of cyanide with four disulfides to displace a thiol anion was evaluated as a spectrophotometric method for determination of cyanide (50). N,N-dimethylformamide is added to the test solution to increase the rate of reaction and improve sensitivity (51). Absorbance of the thiol anion is measured and related to the cyanide concentration over the range of 2.6 to 26 mg/L. Ions of cadmium, copper, iron, mercury, nickel, lead, and zinc interfere at concentrations of 20 mg/L with the determination of 1 mg/L cyanide. EDTA can be added to eliminate interference by all of the cations except copper and mercury. Interference by mercaptans, sulfide, and sulfite can be minimized by adjusting the pH.

Fluorometric Methods--

Demasking reactions--Demasking reactions which form fluorescent compounds have been used for determination of cyanide (52-55). A qualitative test for cyanide based on the demasking of dimethylgloxime by the reaction of cyanide with palladium (II) dimethylgloxime was developed (53). Nickel in the solution reacts with the dimethylgloxime to form the red nickel dimethylgloxime. A fluorescent spot test for detecting cyanide in vapor based on the demasking of oxine from copper (II) oxinate which permits formation of a fluorescent aluminum oxinate compound was proposed (52). A quantitative method for analysis of trace levels of cyanide based on the demasking of 8-hydroxy-5-quinolinesulfonic acid by cyanide from the nonfluorescent potassium bis(5-sulfoxino) palladium (II) was reported (54). The liberated 8-hydroxy-5-quinolinesulfonic acid coordinates with magnesium ions to form a fluorescent chelate, the intensity of which is proportional to the amount of cyanide present. This method has been adapted to the automated determination of cyanide in blood (24). It was found that the demasked 8-hydroxy-7-iodo-5-quinolinesulfonic acid) formed by reaction of cyanide with potassium bis-(7-iodo-5-sulfoxino) palladium (II) forms a stable blue-green color upon addition of a ferric salt (54). The fluorometric method is approximately two orders of magnitude more sensitive than the colorimetric method. Achievement of accurate and precise results with the fluorometric method requires careful control of pH and reaction time. Both methods respond to sulfide, and the colorimetric reaction responds to thiocyanate, thiols, and certain disulfides as well.

The reaction of cyanide with the nonfluorescent bis-palladium-bis-(2,3-diaminonaphthalene-selenium)tetrachloride (DANSe) to demask the fluorescent DANSe was proposed as a method for quantifying cyanide (55). The method can detect concentrations of cyanide as low as 0.6 mg/L.

Other reactions--Fluorescent compounds can be produced by the addition of cyanide to the appropriate anion (56,57), cleavage of certain aromatic rings by cyanide (58), and formation of a specific compound by reaction with cyanide (29). Addition of as little as 0.5 μg of cyanide to quinone monoxime benzene sulfonate ester produces a highly fluorescent compound (57). The fluorescence produced by the reaction of *p*-benzoquinone with cyanide is proportional to the cyanide concentration over the range 0.2 to 50 μg (56). The fluorescence is most intense when dimethylsulfoxide is used as the solvent. No ions interfere in concentrations up to 0.1M.

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A method for determination of cyanide based on cleavage of the pyridine ring of nicotinamide by cyanogen chloride was developed (58). The fluorescent intensity of the cleaved ring is dependent on the pH of the solution and time allowed for reaction, but the method is suitable for determination of 0.3 to 6 mg/L of cyanide.

Catalytic reactions can be used for quantification of cyanide. The oxidation of pyridoxal with oxygen by the catalytic action of cyanide to the fluorescent 4-pyridoxalactone was the basis of a method (59). The finding that the reductant cyanohydrin, formed by reaction of cyanide with p-nitrobenzaldehyde, is capable of reducing a variety of compounds to form a highly colored product has been the basis of several methods (60). The catalytic reaction of cyanohydrin with o-dinitrobenzene to give a blue compound can be used to quantify the cyanide by measuring the change in absorbance over time (60). Cyanide in tissues has been determined by the reaction of cyanohydrin with p-benzoquinone in dimethyl sulfoxide to form a fluorescent product (29). The relationship of fluorescence intensity and cyanide concentration is not linear at low concentrations; thus, the sensititivity is limited to 1 mg/L.

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While fluorometric methods are often more sensitive than colorimetric methods, they have several disadvantages which tend to make them less applicable than other methods for routine analysis of environmental samples. As with colorimetric methods, most fluorometric methods are very sensitive to the pH of the sample solution. Also critical to obtaining reproducible data is the requirement that the fluorescence be measured after a precise interval of time. Frequently the compounds used in fluorescent reactions are unavailable commercially and must be synthesized or purified in the laboratory.

Titrimetric Methods--

The standard method for quantifying cyanide at concentrations greater than 1 mg/L is titration with silver nitrate solution (9,13). The endpoint is detected by the silver-sensitive indicator p-dimethylaminobenzalrhodanine (9,13,61) or by a silver ion-selective electrode (62). Others have titrated the sample with Hg-EDTA using mercury (II) cresol-phthaleincomplexone-iodide as the endpoint indicator (63) and with chloramine-T, dichloramine-T, and lead tetraacetate using a platinum indicator electrode and a reference calomel electrode to detect the endpoint (64). Cyanide in a solution can be determined indirectly by oxidation with chloramine-T or dichloramine-T. An excess of potassium iodide is added to the solution and titrated with sodium thiosulfate (65). Another method is to oxidize the cyanide with bromine, remove excess bromine with phenol, and determine the cyanogen bromide iodometrically (12). The performance of a cation-selective glass electrode and a silver metal electrode were compared for detecting the endpoint in argentometric determination of cyanide (66). It was found that the glass electrode was superior for titration purposes.

Ion-Selective Electrodes--

A cyanide or silver ion-selective electrode can be used to quantify the concentration of free cyanide ion in a solution (67-70). The activity of

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the cyanide ion is determined by the electrode; therefore, the ionic strength of samples must be controlled (68,70). The easiest way to accomplish this is by diluting samples and standards 1:1 with 2M sodium hydroxide which serves to swamp out variations in the original total ionic strength (6,70). Another approach to determining the cyanide concentration with an ion-selective electrode is the method of known additions. A known amount of cyanide is added to a known volume of sample, and the change in electrode potential is used to calculate the cyanide concentration (68). Principal interferences are the sulfide and iodide ions (70). Reported limits of detection range from 0.02 mg/L (68) to 0.3 mg/L (70). The cyanide ion-selective electrode has been used to quantify cyanide in water (71), industrial effluents (68,70,72,73), plant homogenates (73), and fruit and plant distillates (75). A comparison of the determination of cyanide in steelworks effluents by the cyanide ion-selective electrode, argentometric titration, pyridine-pyrazolone colorimetric method, and pyridine-barbituric acid colorimetric method was made (72). The electrode method and argentometric titration gave comparable results for cyanide concentrations greater than 1 mg/L. At lower levels, the pyridine-pyrazolone method tended to give higher results than the other methods. Quantification of cyanide ion by the electrode is a quick, simple method when the concentration is in the optimal range and no interferences are present.

Gas Chromatographic Methods--

Gas chromatography (GC) can be used to quantify cyanide in gases (76), water (1,25,77), blood and urine (27), and alcoholic beverages (6). Celite or Chromosorb coated with 20 percent polyethylene glycol 1500 is satisfactory for separating hydrogen cyanide from water vapor and permanent gases (76). Hydrogen cyanide ranging in concentration from 0.026 to 13 mg/L can be purged from solution and concentrated on a column packed with 19.4 percent dinonyl phthalate on 40/60 mesh firebrick (25). Compounds which are not separated from the hydrogen cyanide and, thus, interfere are trimethylamine, methylamine, diethylamine, methanol, pentanes, and pentenes (25). Another approach is bromination of the cyanide followed by injection of the aqueous solution (1,77) or a diisopropyl ether extract (6) onto a Porapack Q column. The minimum cyanide concentration in the sample which can be detected by the electron capture detector was reported to be 10 μg/L for direct aqueous injection (1,77) and 1 μg/L for the ether extraction procedure (6).

Miscellaneous Instrumental Methods--

Instrumental methods of analysis for cyanide which have received limited use include coulometry (78), polarography (79), and atomic absorption spectrophotometry (AAS) (80). Cyanide at levels ranging from 13 to 260 μg/sample has been determined by coulometric titration with electrogenerated hypobromite (78). The technique is accurate and rapid but very sensitive to slight pH changes. Determination of cyanide ion at levels as low as 2×10^{-14} μg/mL was achieved using a polarographic method (79). The response of a rapidly rotating gold electrode to cyanide ion in lithium hydroxide was used to quantify the cyanide in the aqueous sample. Cyanide can be determined indirectly by AAS. The cyanide is allowed to react with a

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metal, and the resulting cyanide-metal complex is quantified by AAS. Examples of this method include precipitation of cyanide with silver and formation of a dicyano-bis(1,10-phenanthroline) iron (II) complex (80).

Selection of Methods

Methods which appeared to be suitable for adaptation to analysis of solid and semisolid environmental samples for cyanide were selected for empirical evaluation in Phase II. Distillation and microdiffusion can be used to separate free cyanide from a liquid matrix. Distillation has an advantage over diffusion in that heat and catalysts can be applied to aid the breakdown of complex cyanides to permit determination of total cyanide in the sample. Efficient removal of cyanide from relatively large volumes of sample can be achieved with distillation. For these reasons, distillation was selected as one of the separation procedures for evaluation for analysis of solid samples. The other separation procedure chosen was ion-pair extraction. While no references were found for extraction of cyanide as an ion pair, the technique warranted investigation (81).

Selection criteria used in evaluating quantification methods in the literature were analytical range and accuracy of the method, ease of determination, lack of significant interferences, and minimal variation of analytical response as a function of sample pH or temperature. The quantification methods which were evaluated are listed below.

1. The colorimetric method based on Konig synthesis of a dye by the reaction of cyanide with chloramine-T to form cyanogen chloride which subsequently reacts with pyridine-barbituric acid reagent (9).

2. Colorimetric determination based upon formation and extraction of the neutral dicyano-bis(1,10-phenanthroline) iron (II) complex produced through the exchange reaction between tris(1,10-phenanthroline) iron (II) and cyanide ions (46).

3. Ultraviolet spectrophotometric determination based upon the absorbance of the chloranilate ion formed by reaction of mercuric chloranilate with cyanide (49).

4. Ultraviolet spectrophotometric quantification of the tetracyanonickelate (II) anion complex formed by reaction of hydrogen cyanide with ammoniacal nickel chloride (5).

5. Spectrophotometric determination of the thiol anion which is displaced by reaction of N,N-dimethylformamide with cyanide ions (51).

6. Direct determination of cyanide ion activity using an ion selective electrode.

7. GC determination of cyanogen bromide formed by bromination of cyanide in the sample (77).

PHASE II: EVALUATION OF THE METHODS

The goals of this phase were to eliminate nonviable methods and identify necessary modifications of promising procedures. Analysis of a solid or semisolid environmental sample for cyanide was approached as a two-step procedure: separation of cyanide from the sample matrix and quantification of the cyanide. Experiments were performed to evaluate two separation and seven determination procedures. Distillation and solvent extraction as an ion pair complex were the separation procedures studied. Of the seven determination procedures, five were spectrophotometric procedures based on measurement of the absorbance of a compound formed by reaction of a reagent with cyanide. The other procedures were quantification using a cyanide ion-selective electrode and a gas chromatograph equipped with an electron capture detector.

Three solid or semisolid matrices were used for the evaluation of separation procedures. The matrices were selected to cover the range of characteristics encountered in solid and semisolid environmental samples. Lake sediment was used as a representative inorganic matrix. Fish tissue was employed as a matrix of high organic content. Organic domestic sludge from a sewage treatment plant drying pond, the third matrix, was characterized as being an organic material containing significant levels of trace metals.

In all experiments evaluating separation procedures, samples were spiked with a sodium hydroxide solution containing ^{14}C-labeled potassium cyanide. The use of ^{14}C-labeled cyanide permitted determination of recovery achieved by separation procedures by liquid scintillation counting of the ^{14}C. Using this technique avoided the necessity of having to rely on determination procedures designed for analysis of water samples which had not been validated for solid and semisolid samples. The assumption was made that ^{14}C-labeled cyanide responds exactly as unlabeled cyanide to separation and determination procedures. The comparability of liquid scintillation counting and a conventional analytical procedure was demonstrated by using liquid scintillation counting and a cyanide ion-selective electrode to quantify cyanide in 2 sets of 10 replicate samples. The average concentrations of cyanide measured in the first set were 0.75 mg/L ± 0.04 and 0.76 mg/L ± 0.12 with liquid scintillation counting and the ion-selective electrode, respectively. Analysis of the second set yielded 0.77 mg/L ± 0.04 and 0.82 mg/L ± 0.11 using liquid scintillation counting and the ion-selective electrode, respectively.

Separation and determination procedures were evaluated by performing the procedures on seven replicate samples. The percent recovery and standard deviation of the replicates were calculated using the following equations:

$$\text{Percent recovery} = \frac{100\,(O_i)}{T_i}$$

where O_i = observed value

T_i = true value

$$\text{Average percent recovery} = \frac{\sum_{i=1}^{n} P_i}{n}$$

where n = number of samples

P_i = percent recovery of sample i

$$\text{Standard deviation} = \sqrt{\frac{1}{n-1}\left[\sum_{i=1}^{n} P_i^2 - \left(\sum_{i=1}^{n} P_i\right)^2 / n\right]}$$

Modifications were often made of procedures which were determined to be viable. Procedures and modifications of procedures which were judged to be unsatisfactory by initial experiments were not always tested by performing the procedure on seven replicates.

Procedures for Isolation

Isolation procedures were tested on samples of fish tissue, sediment, and sludge. Large quantities of each matrix were obtained and processed as described below to provide material for all evaluation experiments. Fish tissue was prepared for analysis by chopping the edible portions of fresh fish into small pieces and homogenizing in a blender. The tissue was stored in a freezer until needed. Lake sediment was dried at 103°C and then cleaned of large pieces of debris such as sticks, leaves, and shells. Sludge was dried at 103°C, pulverized with a mortar and pestle to generate a powdery matrix, and stored at room temperature.

A procedure was developed for spiking samples of the solid matrix prior to analysis. Solid and semisolid samples were moistened with sodium hydroxide solution and then spiked with a small volume of sodium hydroxide solution containing radiolabeled potassium cyanide. Sludge and sediment samples were held at room temperature for approximately 16 hours to allow time for adsorption of the cyanide to the solid particles. Fish tissue samples were held for 16 hours at 4-6°C to prevent decomposition of the tissue. Control samples were prepared with each set of solid samples by adding aliquots of the sodium hydroxide solution and cyanide spiking solution to an appropriate volume of water. Controls were treated by the same procedure as the solid samples and were identical to the solid samples except that they contained no solid matrix.

Liquid scintillation counting was used to determine the concentration of ^{14}C in all solutions. Aliquots of solutions containing ^{14}C were counted at various steps of the procedures to determine recovery of cyanide. The concentration of radiolabeled cyanide was determined by placing 0.5 mL or 1.0 mL of solution in a glass vial containing 10 mL of liquid scintillation cocktail and counting with a liquid scintillation counter. Three replicate aliquots of each sample were always counted, and the average was reported. Blanks were prepared by taking an unspiked sample or control through a separation procedure. The levels of ^{14}C found in the blanks were never above expected background.

Distillation--

Before work with solid samples was initiated, experiments were performed to verify that the distillation apparatus and the experimental techniques used would give high recovery of cyanide distilled from water. The distillation apparatus consisted of a one-liter round bottom distillation flask equipped with a condenser, air inlet tube, and absorber tube. A vacuum system was used to pull air into the flask through the air inlet tube and out through the absorber tube. It was determined that the air flow rate is a significant factor affecting the recovery of cyanide. Recoveries above 80 percent were obtained when the air flow rate was great enough to produce a layer of foam 2 cm high on the top of the sodium hydroxide solution in the absorber tube.

The effect of holding time between spiking and distilling the samples on the recovery of cyanide was briefly investigated. The relationship of recovery of cyanide to the length of holding time between spiking and distilling was studied by spiking four sediment samples and four controls containing no sediment as described above. Distillation of two samples and two controls was initiated 0.5 hour after completion of spiking. The remainder of the samples was held 16 hours and then distilled. The purpose of this experiment was to determine if the percent accounted for was lower for samples held overnight because of loss of cyanide from the sample and flask. As shown in Table 1, the percent accounted for is similar for samples and controls held for 0.5 hour and 16 hours. Recovery of cyanide from samples held for 16 hours may be lower than that of samples held for 0.5 hour because of increased adsorption of cyanide to the solid matrix, but cyanide does not appear to be lost from the system.

Two basic procedures were evaluated for separating cyanide from solid and semisolid environmental samples. The procedures were distillation and ion pair solvent extraction. Several variations of distillation were tried. These involved stirring the sample with a solution of sodium hydroxide prior to distillation, stirring the sample with sulfuric acid and reagents during distillation, or substituting mercuric or cuprous chloride for the magnesium chloride refluxing reagent.

TABLE 1. COMPARISON OF RECOVERY OF CYANIDE FROM SEDIMENT HELD 0.5 HR AND 16 HR BEFORE DISTILLING

Sample	Number of replicates	Holding time between spiking and distilling (hr)	Percent recovered by 1 hr distillation	Percent remaining in flask	Total percent accounted for
Sediment	2	0.5	86 ± 1	16 ± 0	102 ± 1
Sediment	2	16	78 ± 0	24 ± 0	102 ± 0
Control	2	0.5	86 ± 8	14 ± 0	100 ± 8
Control	2	16	94 ± 2	16 ± 0	110 ± 2

Experiments designed to assess recovery of cyanide from solid and semisolid samples by distillation were performed. Ten-gram portions of sludge were spiked with 100 µg of radiolabeled cyanide as potassium cyanide in a sodium hydroxide solution. Ten-gram samples of fish tissue were spiked with 50 µg of radiolabeled cyanide. Controls were prepared in the same manner but contained no sludge or fish tissue. Immediately prior to distillation, 250 mL of distilled water, 10 mL of 5M magnesium chloride solution, and 12 mL of concentrated sulfuric acid were added to the sludge or fish tissue in the distillation flask. The flask was attached to an absorber tube filled with 50 mL of 1.25M sodium hydroxide solution. Air was bubbled through the distillation system at a rate great enough to maintain about 2 cm of foam on the surface of the sodium hydroxide solution in the absorber tube. It was desirable to know the time period required for recovery of all of the cyanide from the samples. A set of samples was distilled for 30 minutes, fresh sodium hydroxide solution was placed in the absorber tube, and the samples were distilled for a second 30-minute period. The sodium hydroxide solution was again replaced, and the samples were distilled for a second hour. The average percentage of cyanide recovered from each matrix for each distillation period is shown in Table 2. Recovery after 1 hour of distillation ranged from 35 percent for fish tissue and 39 percent for sludge to 90 percent for controls. An additional 9 percent of the cyanide was recovered from samples of sludge and fish tissue during the second hour of distillation.

The distillation procedure was modified to increase the recovery of total cyanide from solid samples. Five- or ten-gram portions of fish tissue, sediment, and sludge were spiked with 50 to 200 µg of radiolabeled cyanide as potassium cyanide. The samples were held overnight and then stirred with 100 mL of 2M or 4M sodium hydroxide solution for 1 hour. The mixture was transferred to a distillation flask, and the volume was brought up to 500 mL with distilled water. Twenty-five or fifty milliliters of concentrated sulfuric acid and 20 mL of 5M magnesium chloride solution were added to the distillation flask. The mixture was boiled gently, and a flow of air was bubbled through the system to carry hydrogen cyanide from the mixture to an absorber tube containing 50 mL of 1.25M sodium hydroxide solution. The samples were distilled for 1 hour, the sodium hydroxide solution in the absorber tube was replaced with fresh solution, and the distillations were continued for a second hour. The average percentages of cyanide recovered by the sodium hydroxide stirring and distillation procedure are shown in Table 2. Recovery tended to be about the same or slightly lower than recovery achieved by distillation alone. Recovery of cyanide from fish tissue was 28 percent for 1 hour of distillation and 36 percent for 2 hours. Thirty-eight and 43 percent were recovered from sludge by 1 and 2 hours of distillation, respectively. The recovery of cyanide from sediment was 64 percent after 1 hour with no increase after 2 hours of distillation.

TABLE 2. PERCENTAGES AND STANDARD DEVIATIONS OF CYANIDE RECOVERED FROM THREE MATRICES BY DISTILLATION PROCEDURES

Matrix	Duration of procedure (hr)	Distillation	Distillation preceded by stirring with NaOH solution	Distillation with stirring
Fish tissue	1	35 ± 4	28 ± 4	--
	2	44 ± 4	36 ± 4	--
Sediment	1	--	64 ± 15	69 ± 15
	2	--	63 ± 16	71 ± 10
Sludge	0.5	30 ± 1	--	--
	1	39 ± 6	38 ± 6	43 ± 6
	2	--	43 ± 4	52 ± 7
Controls (water)	0.5	76	--	--
	1	90 ± 2	85 ± 7	84 ± 20
	2	93	--	103 ± 4

Another set of experiments was performed to determine whether higher recoveries could be obtained by stirring the slurried sample during distillation. Stirring prevents sample particles from settling to the bottom of the distillation flask and reduces bumping of the flask. Ten-gram portions of dry sediment were spiked with 47 μg of radiolabeled cyanide as potassium cyanide in a sodium hydroxide solution. The sludge samples were prepared in a similar manner, but 5-g portions of dry sludge were spiked with 47 μg of radiolabeled cyanide. Controls were prepared for the sludge and sediment samples by adding the sodium hydroxide solution and radiolabeled potassium cyanide to an empty distillation flask. Immediately prior to distillation, 500 mL of distilled water, 25 mL of concentrated sulfuric acid, and 20 mL of 2.5M magnesium chloride solution were added to the samples. The flasks were connected to absorber tubes filled with 50 mL of

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1.25M sodium hydroxide solution, and the samples were stirred with a magnetic stirring bar and distilled for 1 or 2 hours.

Recovery of cyanide from the sediment and sludge samples was slightly higher than that measured in previous experiments as shown in Table 2. Seventy-one percent of the cyanide was recovered from the sediment and 52 percent from the sludge by 2 hours of stirring and distilling. The greatest advantage of this procedure is that stirring tends to decrease bumping of the distillation flasks to make distillation of a larger size sample feasible.

A series of experiments was performed to determine whether substituting solutions of cuprous chloride or mercuric chloride for the magnesium chloride refluxing reagent would improve the recovery of cyanide. Five grams of sludge or 10 g of sediment or tissue were spiked with 50 µg of radiolabeled cyanide as potassium cyanide in a sodium hydroxide solution. A 500-mL volume of distilled water; a specific volume of magnesium chloride, cuprous chloride, or mercuric choloride; and 25 ml of concentrated sulfuric acid were added to the samples. The flask was connected to an absorber tube filled with 50 mL of a 1.25M sodium hydroxide solution, and samples were distilled for 1 hour unless otherwise noted.

Details of the experiments and the recovery of cyanide as determined by liquid scintillation counting are presented in Table 3. Comparison of the average percentage of cyanide recovered using the magnesium chloride reagent with that using cuprous chloride or mercuric chloride reagents showed no significant difference. Distillation for a second hour increased the recovery by an average of 4 percent for sediment, 6 percent for sludge, and 17 percent for fish tissue.

Solvent Extraction--

In addition to distillation, solvent extraction was evaluated as a procedure for separating cyanide from solid matrices. Preliminary experiments to evaluate the procedure were performed on samples of dilute sodium hydroxide solution containing ^{14}C-labeled and unlabeled potassium cyanide. Ten-milliliter aliquots of solution containing 5 µg of cyanide and 1-mL aliquots of 0.001M ammonium hydroxide were place in test tubes. The ammonium-cyanide ion pair was extracted from aqueous solution with hexane or methylene chloride. Recoveries of cyanide were determined by liquid scintillation counting of the extraction solvent and the samples before and after extraction. Less than 1 percent of the cyanide was extracted by either solvent. No further work was performed with this procedure. Initial experiments showed it to be unsatisfactory, and problems with extraction of cyanide as an ion-pair from solid samples were anticipated.

Procedures for Quantification

Seven analytical procedures for quantification of cyanide were selected from the literature for evaluation. Criteria used for selection were analytical range of the procedure, lack of significant interferences, and minimal variation of analytical response as a function of pH, temperature,

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TABLE 3. RECOVERY OF CYANIDE FROM FISH TISSUE, SEDIMENT AND SLUDGE BY DISTILLATION WITH SEVERAL REFLUXING REAGENTS

Matrix	Number of replicates	Volume of reagent	Average percent recovered ± standard deviation 1 hr	Average percent recovered ± standard deviation 2 hr	Average percent recovered ± standard deviation by 1 hr of distillation using only $MgCl_2$
Fish tissue	3	40 ml of 2.5M $MgCl_2$ + 10 ml of 0.25M $HgCl_2$	28 ± 4	45 ± 1	35 ± 4
Sediment	3	10 ml of 0.2M CuCl	61 ± 10	65 ± 9	65 ± 17*
Sludge	3	40 ml of 2.5M $MgCl_2$ + 10 ml of 0.25M $HgCl_2$	34 ± 3	40 ± 2	37 ± 8

*Samples were stirred with sodium hydroxide solution prior to distillation.

and reaction time. Evaluation of determination procedures was performed using solutions of potassium cyanide prepared in dilute sodium hydroxide solution. Solutions were prepared fresh daily by dilution from a stock solution of 1000 mg/L cyanide. The stock cyanide solution was standardized by titration with a standard solution of silver nitrate. Evaluation of the procedures was based on precision and accuracy data generated by analysis of aqueous solutions of potassium cyanide. Seven replicate samples of concentration approximately ten times the limit of detection were analyzed by each procedure. The average percent recovery and standard deviation were calculated for all of the procedures except quantification by GC and are presented in Table 4. Complete information on reagents and steps of the procedures, which are listed on pages 14 and 15, are included in Appendix A.

The GC procedure was not thoroughly evaluated, because of the poor recovery of the ion-pair extraction procedure prescribed above and the numerous problems encountered with the GC procedure itself. However, preliminary evaluation was made of the GC quantification procedures which consists of 2 steps, formation of cyanogen bromide and analysis by GC.

Cyanide in samples which were to be analyzed by GC was reacted with bromine to form cyanogen bromide which was then extracted into an organic solvent. The following procedure for bromination (77) and extraction of cyanide in aqueous samples was evaluated. Replicate 50-mL aliquots of 0.1M potassium hydroxide solution containing 5 μg of radiolabeled cyanide as potassium cyanide were treated with bromine and extracted by the following procedure. The pH of the solution was adjusted to 4 or 5 with 20 percent phosphoric acid. Cyanogen bromide was formed by adding saturated bromine water to the solutions drop-by-drop until a yellow color persisted. An excess of 5 drops was added; the sample was shaken to mix and held for 5 minutes. A sufficient volume of a 5 percent phenol solution was added to each sample to react with the excess bromine. Samples were kept in closed flasks and chilled in an ice bath to minimize loss of volatile forms of cyanide. Four solvents, 3-pentanone, methyl isobutyl ketone, isopropyl ether, and propionaldehyde, were evaluated for the extraction of cyanogen bromide.

The percent recovery and standard deviation as determined by liquid scintillation counting for extraction of three replicate samples by each solvent are listed in Table 5. Recoveries ranged from 34 to 55 percent. Aliquots of the sample solution taken after the addition of the reagents were subjected to liquid scintillation counting to determine the amount of cyanide lost during the reaction. Only 4 percent of the cyanide was lost from the sample. Thus, the low recoveries of cyanide are the result of low extraction efficiency, not loss of cyanide from the sample.

Aqueous samples of unlabeled potassium cyanide were brominated and extracted with methyl isobutyl ketone. These extracts were analyzed using an electron capture gas chromatograph equipped with a column packed with Porapak Q, 80-100 mesh. The oven temperature was maintained at 110°C. Levels of cyanogen bromide as low as 1 ng on column were detected by this technique. Numerous problems, including water in the extracts and contamination of the methyl isobutyl ketone, were encountered.

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TABLE 4. RESULTS OF EVALUATION OF PROCEDURES FOR DETERMINATION OF CYANIDE

Method	Range (mg/L)	Average ± standard deviation (mg/L)	Average percent recovery
Ultraviolet spectrophotometric quantification of the tetracyanonickelate (II) anion complex formed by reaction of hydrogen cyanide with ammoniacal nickel chloride.	0.2 - 10	3.7 ± 0.4	94
Spectrophotometric determination of the thiol anion which is displaced by reaction of 5,5'-dithiobis (2-nitrobenzoic acid) with cyanide ion.	0.11 - 3.0	1.5 ± 0.04	84
Colorimetric determination based on Konig synthesis of a dye by the reaction of cyanide with chloramine-T to form cyanogen chloride which subsequently reacts with pyridine-barbituric acid reagent.	0.02 - 0.60	0.2 ± 0.2	97
Colorimetric determination based upon formation and extraction of the neutral dicyano-bis(1,10-phenanthroline) iron (II) complex produced through the exchange reaction between tris (1,10-phenanthroline) iron (II) and cyanide ions.	0.2 - 1.0	1.0 ± 0.1	109
Ultraviolet spectrophotometric determination based upon the absorbance of the chloranilate ion formed by reaction of mercuric chloranilate with cyanide.	0.2 - 4.0	2.0 ± 0.14	88
Determination of cyanide ion activity using an ion selective electrode.			
Direct	1 - 100	10 ± 1	96
Indirect	0.06 - 1.00		--

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TABLE 5. PERCENT RECOVERY OF CYANIDE BY SOLVENT EXTRACTION AS CYANOGEN BROMIDE

Solvent	Percent recovered ± standard deviation
3-pentanone	50 ± 2
methyl isobutyl ketone	55 ± 0
isopropyl ether	34 ± 1
propionaldehyde	47 ± 2

The most sensitive quantification procedure is colorimetric determination based on the reaction of cyanogen chloride with pyridine-barbituric acid reagent (Table 4). The pyridine-barbituric acid reagent procedure is easy to conduct, and the only instrumentation required is a spectrophotometer. The reaction of cyanide with chloramine-T to produce cyanogen chloride and the subsequent reaction with pyridine-barbituric acid reagent to form the dye are dependent on the temperature and pH of the sample solution. Several compounds, particularly sulfide, interfere with the determination. The remainder of the colorimetric determination procedures are not as sensitive as the pyridine-barbituric acid procedure. The cyanide ion-selective electrode provides a fast procedure for quantifying cyanide in aqueous solutions, but it is subject to interferences and has a relatively high detection limit.

Selection of Method

Distillation with stirring followed by quantification by the pyridine-barbituric acid procedure was selected as the best method for determination of cyanide in solid and semisolid samples. Stirring the sample with a magnetic stirrer during distillation allows a larger size sample to be used and reduces bumping of the distillation flask encountered with certain solid matrices. Whether or not stirring is necessary depends on the nature of the matrix and the size of sample. The expected cyanide concentration and the desired detection limit should be considered when deciding on the appropriate size of sample for distillation. The pyridine-barbituric acid colorimetric procedure is sensitive to low concentrations of cyanide and is subject to minimal interferences.

PHASE III: OPTIMIZATION OF THE METHOD

During optimization studies, the most promising isolation and quantification procedures for cyanide were joined to form a single method for analysis of solid and semisolid environmental samples for cyanide. Cyanide was isolated from the solid or semisolid sample matrix by distillation. The cyanide in the distillate was quantified by the pyridine-barbituric acid

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procedure. The response of the quantification procedure to volatile sulfur compounds, expected to be the most significant interference (82), was investigated. An absorber tube containing a lead acetate solution was utilized for removal of volatile sulfur-containing compounds from the distillate. Removal of the interferences and recovery of the cyanide were monitored using the pyridine-barbituric acid colorimetric procedure and the cyanide ion-selective electrode. After experimental conditions had been optimized, replicate samples of fish tissue were spiked with cyanide and analyzed to determine the precision and accuracy of the method. This information and the optimized method are presented below.

The method developed for analysis of solid and semisolid environmental samples for cyanide consisted of distillation of hydrogen cyanide from an acidified slurry of the sample into sodium hydroxide solution followed by quantification of the cyanide in the solution by the pyridine-barbituric acid colorimetric method. Sulfur-containing compounds which are distilled from the sample and absorbed by the sodium hydroxide solution are known to interfere with the colorimetric determination (82). The use of a trap filled with lead acetate solution (83) was evaluated for removal of volatile sulfur compounds from the distillate. A trap consisting of an open-ended glass tube inserted into a tube containing 25 mL of 0.08M lead acetate solution was placed in series between the distillation flask and the absorber tube containing sodium hydroxide solution (Figure 2).

Samples of wet ground fish tissue weighing 10 g were placed in distillation flasks, moistened with sodium hydroxide solution to insure basic pH conditions, and spiked with 1 mL of a sodium hydroxide solution containing 100 µg/L of potassium cyanide. The spiked samples were held for approximately 16 hours at 4-6°C. Immediately prior to distillation, 500 mL of distilled water, 50 mL of concentrated sulfuric acid, and 20 mL of magnesium chloride solution were added to each sample. The samples were boiled for 2 hours to distill the cyanide which was absorbed in 35 mL of 1.8M sodium hydroxide solution. Stirring the samples during distillation was not necessary because of the reduction in sample size from 25 g to 10 g. The cyanide in the distillate was quantified by both the pyridine-barbituric acid colorimetric procedure and the cyanide ion-selective electrode. The colorimetric procedure gives a negative response to sulfur compounds, and the cyanide ion-selective electrode gives a positive response. The two sets of results were compared to detect the presence of interferences.

The percentages of cyanide recovered from samples of spiked fish distilled with and without the presence of lead acetate traps are presented in Table 6. The average recoveries of cyanide from samples of fish tissue distilled in the presence of traps containing lead acetate solution as determined by the ion-selective electrode and the pyridine-barbituric acid procedure are comparable. The average recovery of 54 percent is similar to the recovery from fish tissue previously obtained. The average recovery of cyanide from samples distilled without the use of lead acetate solution-filled traps was determined to be 295 percent by the electrode and 25 percent by the colorimetric procedure. This difference is probably due to the presence of sulfur compounds in the distillate. Thus, it is necessary to

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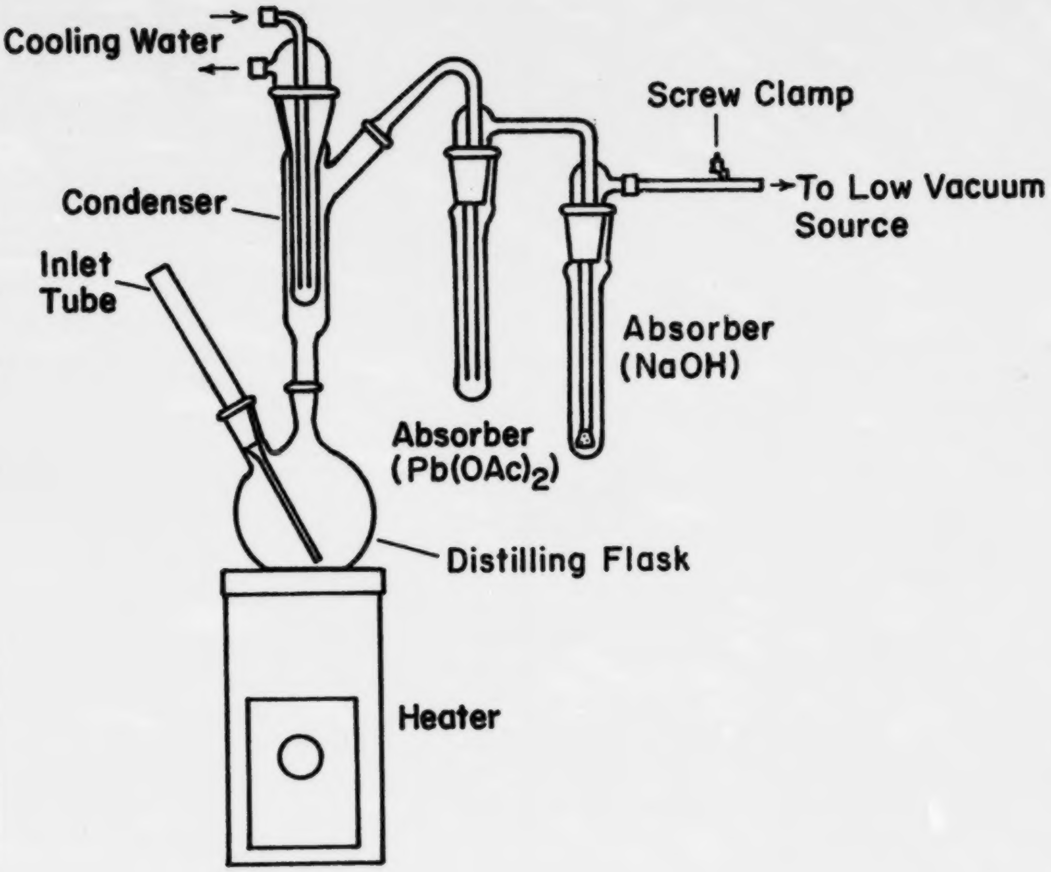
Cooling Water
Condenser
Inlet Tube
Screw Clamp
To Low Vacuum Source
Absorber (NaOH)
Absorber $(Pb(OAc)_2)$
Distilling Flask
Heater

Figure 2. Cyanide distillation apparatus equipped with a lead acetate-filled absorber tube.

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use the lead acetate traps when distilling solid environmental samples to remove potential interferences.

TABLE 6. COMPARISON OF PERCENTAGES OF CYANIDE RECOVERED FROM FISH TISSUE SPIKED WITH 100 MICROGRAMS OF KCN DISTILLED WITH AND WITHOUT A LEAD ACETATE TRAP AS DETERMINED BY A CYANIDE ION-SELECTIVE ELECTRODE AND THE PYRIDINE-BARBITURIC ACID PROCEDURE

Replicate number	Lead acetate traps used	Ion-selective electrode	Pyridine-barbituric acid procedure
1	yes	51	50
2	yes	43	35
3	yes	78	89
4	yes	47	35
5	yes	56	55
average ± standard deviation		55 ± 14	53 ± 22
6	no	77	26
7	no	295	43
8	no	514	5
average ± standard deviation		295 ± 218	25 ± 19

Experiments were performed to determine the precision and accuracy of the method for analysis of solid and semisolid environmental samples for cyanide. Seven replicate 10-g portions of fish tissue were distilled, and the cyanide concentration was determined by the optimized method. Three groups consisting of seven 10-g replicates of the fish tissue were spiked with 10, 25, and 100 μg of cyanide as potassium cyanide. The samples were analyzed, and the results are presented in Table 7. The average percent recoveries were 94 ± 1.4, 95 ± 1.2, and 82 ± 5.6, respectively, for samples spiked with 10, 25, and 100 μg of cyanide. The increase in recovery can be attributed to improved technician skill.

The sensitivity of the method is dependent on the size of sample distilled and is based on the concentration of cyanide which has an absorbance reading of 0.02 at 578 nm. A detection limit of 0.5 μg/g can be achieved by using a 10-g sample.

The method for analysis of solid and semisolid environmental samples for cyanide is presented in Appendix B. Briefly, solid samples are blended or ground and placed in a distillation flask. Magnesium chloride solution

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and sulfuric acid are added to the sample, and hydrogen cyanide is distilled into an absorber tube containing sodium hydroxide solution. An absorber tube containing lead acetate solution is placed in series between the distillation flask and the absorber tube. The sample can be stirred while the distillation is being performed using a combination magnetic stirrer-hot plate. Stirring reduces bumping and, therefore, makes the use of a larger sample feasible. The cyanide absorbed by the sodium hydroxide solution is quantified by the pyridine-barbituric acid colorimetric procedure.

TABLE 7. MICROGRAMS OF CYANIDE RECOVERED FROM SPIKED AND UNSPIKED SAMPLES OF FISH TISSUE

Replicate number	μg of cyanide added to sample 0	10	25	100
1	0	9	23	75
2	0	7	23	80
3	0	11	23	90
4	0	9	23	77
5	0	10	23	82
6	0	11	25	89
7	0	9	26	82
average μg recovered	0	9.4	23.7	82.1
standard deviation	0	1.4	1.2	5.6
coefficient of variation		15%	5%	7%
percent of phenol recovered		94%	95%	82%

PHASE IV: VALIDATION OF THE METHOD

The method developed for analysis of solid and semisolid environmental samples for cyanide was validated by analysis of a variety of matrices. The method consists of two steps: distillation of hydrogen cyanide from an acidified slurry of the solid or semisolid sample and quantification of the cyanide by the pyridine-barbituric acid procedure. Volatile sulfur-containing compounds which might interfere with quantification of cyanide are removed by bubbling the distillate through a solution of lead acetate. Details of the method are presented in Appendix B.

The method for determination of cyanide in solid and semisolid environmental samples was validated by the analysis of samples of ten matrices:

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1. bottom sediment from a lake
2. industrial sludge
3. industrial solid wastes from a disposal site
4. fish tissue
5. microinvertebrates (shrimp)
6. algae
7. soil contaminated by chemicals from the disposal of industrial sludges
8. growing vegetation
9. slags from industrial processing which are disposed of in a landfill
10. fly ash from a commercial incinerator which is disposed of in a landfill

One sample of each matrix was obtained, and six 10-g portions of each sample were frozen to preserve the cyanide. Two portions of each sample matrix were analyzed by the developed method to determine the concentrations of cyanide in the samples. The four remaining portions of each matrix were removed from the freezer, spiked with a sodium hydroxide solution containing potassium cyanide, and analyzed. Two portions of each matrix were spiked with 26 µg of cyanide, and the remaining two were spiked with 80 or 200 µg of cyanide to give spiked cyanide concentrations of 2.6, 8.0, and 20 µg/g, respectively. Some of the environmental samples contained cyanide, and, thus, the total cyanide concentration of these samples was greater than the spiked concentration alone (Table 8).

The concentrations of cyanide recovered from each sample are presented in Table 8. The recovery of cyanide from the 40 spiked samples ranged from 69 to 112 percent, with an average of 90 ± 10 percent. Recovery did not vary with the concentration of cyanide over the range of 2.6 to 20 µg/g. Twenty samples were spiked with 2.6 µg/g of cyanide. The average percent recovery of cyanide from these samples was 90 ± 10. Average percentages of cyanide recovered from 12 samples spiked with 8.0 µg/g and eight samples spiked with 20 µg/g were 89 ± 13 and 90 ± 11, respectively.

The quality of the data was verified by a rigid quality control program. A calibration curve was prepared each day using standard solutions of potassium cyanide. These standards were not distilled. A reagent-glassware blank consisting of 500 mL of distilled water was distilled and analyzed daily. Performance of the distillation apparatus and quantification procedure was checked daily by distillation and analysis of one quality control sample for each group of four environmental samples. The quality control samples consisted of 500 mL of distilled water spiked with a solution of potassium cyanide to give a concentration of 50 to 400 µg/L. The recoveries of cyanide from the quality control samples are presented in Table 9. The average percent of cyanide recovered from 500-mL volumes of distilled water containing from 25 to 200 µg of cyanide was 88 ± 7. This compares well with the average percent recovery of 89 ± 10 from the 40 spiked environmental samples.

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TABLE 8. CONCENTRATIONS OF CYANIDE ADDED TO AND RECOVERED FROM SOLID AND SEMISOLID ENVIRONMENTAL SAMPLES

Matrix	Concentration added (μg/g)	Concentration recovered (μg/g)	Percent recovery	Average percent recovery
bottom sediments	0	ND		84 ± 6
	0	ND		
	2.6	2.3	88	
	2.6	2.0	77	
	8.0	7.2	90	
	8.0	6.4	80	
industrial sludge	0	1.8		84 ± 12
	0	1.4		
	2.6	3.6	77	
	2.6	4.2	100	
	20	16	72	
	20	19	87	
industrial solid waste	0	ND		94 ± 11
	0	ND		
	2.6	2.3	88	
	2.6	2.3	88	
	20	18	90	
	20	22	110	
industrial processing slag	0	ND		94 ± 3
	0	ND		
	2.6	2.5	96	
	2.6	2.5	96	
	20	19	95	
	20	18	90	
incinerator fly ash	0	0.9		95 ± 7
	0	1.0		
	2.6	3.6	100	
	2.6	3.6	100	
	20	18	85	
	20	20	95	
contaminated soil	0	ND		103 ± 12
	0	ND		
	2.6	2.2	85	
	2.6	2.9	112	
	8.0	8.6	108	
	8.0	8.6	108	

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Table 8 (Continued).

Matrix	Concentration added (μg/g)	Concentration recovered (μg/g)	Percent recovery	Average percent recovery
fish tissue	0	ND		94 ± 6
	0	ND		
	2.6	2.5	96	
	2.6	2.3	88	
	8.0	8.2	102	
	8.0	7.3	91	
microinvertebrates (shrimp)	0	ND		92 ± 6
	0	ND		
	2.6	2.3	88	
	2.6	2.6	100	
	8.0	7.4	92	
	8.0	6.9	86	
algae	0	ND		76 ± 1
	0	ND		
	2.6	2.0	77	
	2.6	2.0	77	
	8.0	6.1	76	
	8.0	6.1	76	
vegetation	0	ND		84 ± 10
	0	ND		
	2.6	2.3	88	
	2.6	2.3	88	
	8.0	7.1	89	
	8.0	5.5	69	

ND = not detected above detection limit of 0.5 μg/g

TABLE 9. RECOVERY OF CYANIDE FROM AQUEOUS QUALITY ASSURANCE SAMPLES

Sample	μg of cyanide in 500 ml	μg of cyanide recovered	Percent recovery
1	25	20	80
2	26	25	96
3	26	22	85
4	26	26	100
5	200	174	87
6	200	158	79
7	26	23	88
8	80	75	94
9	26	25	96
10	80	68	85
11	80	72	90
12	26	22	85
13	26	24	92
14	80	60	75
15	80	67	84
average ± standard deviation			88 ± 7

SECTION 4

METHOD FOR ANALYSIS OF SOLID AND SEMISOLID ENVIRONMENTAL SAMPLES FOR TOTAL PHENOLIC COMPOUNDS

INTRODUCTION

The literature was searched for methods for analysis of any matrix for total phenolic compounds. Numerous publications were located using computerized and manual literature searching techniques. The majority of the references discussed analysis of aqueous samples. Limited information was found on analysis of solid or semisolid matrices. During Phase I, methods which were appropriate for application to the analysis of solid and semisolid environmental samples were selected for empirical evaluation. Selection criteria used in evaluating the methods in the literature were accuracy and analytical range of the methods, lack of significant interferences, minimal sensitivity to changes in sample pH or temperature, and ease of performing the determination.

Evaluation of methods of analysis for total phenolic compounds was approached as a two-step procedure. First, the phenolic compounds were isolated from the solid or semisolid matrix. Solvent extraction and steam distillation were the isolation procedures evaluated. Radiolabeled phenolic compounds were added to solid and semisolid samples, and recovery by the isolation procedures was monitored using liquid scintillation counting. Once the phenolic compounds are in a solution, the concentration can be determined. Three quantification procedures were tested:

1. Phenol + 4-aminoantipyrine → antipyrine dye
2. Phenol + 3-methyl-2-benzothiazolinone hydrazone → azo dye
3. Bathochromatic shift occurring upon ionization of the OH-group of phenol

The quantification procedures were evaluated by analysis of solutions of phenol in distilled water. The sensitivity, analytical range, precision, and accuracy of each method were compared. Solvent extraction was selected as the isolation procedure because it recovers a greater percentage of phenolic compounds than distillation. Measurement of the change in absorbance due to ionization of the hydroxyl group of phenol using the ultraviolet ratio spectrophotometer most sensitive procedure for phenol in distilled water. However the 4-aminoantipyrine method was chosen because it has been shown to work on a large variety of aqueous environmental matrices, and the results can be compared to an abundance of accumulated data.

In the optimization phase, solvent extraction and the 4-aminoantipyrine procedure were joined to form a method for analysis of solid and semisolid environmental samples for total phenolic compounds. Experiments were performed using environmental samples spiked with phenol to determine optimal analytical conditions.

The method was validated by analysis of samples of ten solid or semisolid environmental matrices. The samples were spiked with two levels of phenol and analyzed. Precision, accuracy, and sensitivity of the method are discussed below.

PHASE I: SELECTION OF METHODOLOGY FROM THE LITERATURE

The literature was searched for methods of analysis of any type of matrix for phenolic compounds. Computerized retrieval techniques were used for searching two data bases, CA CONDENSATES/CASIA and TOXLINE. Chemical abstracts for the years 1967 to 1970 and Government Reports and Announcements Index published by the National Technical Information Service for the years 1968 to 1977 were searched manually. Approximately 71 papers of interest were identified by these methods. References cited in the reviewed literature were also obtained.

Methods of Isolation

Samples which are to be analyzed for phenolic compounds frequently contain other materials which may contribute to the turbidity or color of the sample or interfere with the determination in other ways. Three basic techniques are used to separate phenolic compounds from an aqueous matrix. These are distillation, solvent extraction, and liquid chromatography.

Steam Distillation--

Distillation of wastewater samples prior to analysis is recommended by the U.S. EPA (9) and the American Public Health Association (13) to separate the phenolic compounds from nonvolatile components of the sample which could interfere with the determination. The phenolic compounds are distilled from an acidified sample at a constant rate. Phenolic compounds volatilize gradually; therefore, the entire sample must be distilled to ensure complete recovery of all the phenolic compounds (9). Recovery of 80 and 90 percent of the volatile phenolic compounds during steam distillation and normal distillation, respectively, has been reported (84). While distillation serves to separate phenolic compounds from nonvolatile components of the sample, it does not concentrate the phenolic compounds.

Solvent Extraction--

Solvent extraction can be used to separate and concentrate phenolic compounds from the sample matrix. Solvents which have been used to extract phenols from aqueous solutions include dichloromethane (85), petroleum ether (86), chloroform (87,88), toluene (89), dimethylformamide (90), and tributylphosphate (91). A potassium hydroxide solution has been used to extract phenolic compounds from petroleum products (92). To concentrate the phenolic compounds further, the pH of the caustic extract is adjusted to less than seven and the phenols are extracted with dimethylether. The dimethylether is evaporated, and the residue is dissolved in acetic acid. Refinery wastewater samples at pH 12 have been extracted with carbon tetrachloride to remove oil and then extracted at pH 5 with tributylphosphate to recover the phenols as a concentrate (91). Phenolic compounds can be separated from other organic compounds in wastewater by converting the phenols to their salts by the addition of sodium hydroxide (93). The organic compounds are removed from the sample by extraction with a 1:1 mixture of ether-petroleum ether. After separation of the phases, the aqueous phase is acidified by addition of a mineral acid to convert the phenolic salts to free phenols.

Liquid Chromatography--

Ion-exchange, adsorption, and gel chromatography can be used to separate phenolic compounds from a sample matrix. The macroreticular anion exchange resin Amberlyst A-26 and the porous polystyrene-divinyl benzene copolymer XAD-2 (94), as well as the cation exchange resins Amberlite IR 112, Amberlite IRC 50, and Dualite C 25 (95), have been used to adsorb phenolic and other organic compounds from aqueous samples. A method for separating phenolic compounds from a sample matrix based on adsorption of phenolate ions from alkaline solution on the A-26 macroporous anion-exchange resin in the hydroxyl form was developed (96). The adsorbed phenolate ions are converted to the molecular form by washing the column with hydrochloric acid and are eluted from the column with an acetone-water solution. The average recovery of eight phenols from a spiked tap water sample was 97.7 percent (96).

Phenolic compounds can be removed from water by adsorption to carbon or Sephadex gels (97). Because the adsorption capacity of activated carbon is limited, this technique is better suited to sampling water which is clean than water which contains high concentrations of organic compounds (98). The usefulness of the carbon adsorption technique is limited by the adsorption efficiency of 94 percent and desorption efficiency of 22 percent (99). Sephadex gels effectively separate free phenols from high molecular weight proteins and phenolic polymers (100).

Methods of Quantification

Spectrophotometric Methods--

A method for quantification of total phenolic compounds should be able to determine ortho-, meta-, and para-substituted phenolic compounds. The primary shortcomings of many colorimetric methods are that they lack sensitivity to some phenolic compounds (101,102) and produce complexes which absorb at different wavelengths (92,103-105). The five most widely used methods for determining trace levels of phenolic compounds in water were reviewed (106), and it was concluded that the 4-aminoantipyrine procedure is the fastest, most precise, and most accurate when compared to the following spectrophotometric methods: Gibbs' dibromoquinone chlorimide, nitrosophenol, infrared, and ultraviolet.

The 4-aminoantipyrine method is currently the most widely recommended method for analysis of water and wastewater samples (9,13). It is based on the formation of antipyrine dyes by the reaction of phenolic compounds with 4-aminoantipyrine in the presence of an alkaline oxidizing agent (107-109). Compounds which have an open para position or which have groups such as halogen, carboxyl, sulfonic acid, hydroxyl, or methoxyl in the para position are detected (106). Phenolic compounds in which the para position is blocked by an aryl, alkyl, nitro, nitroso, benzoyl, or carbonyl group are not determined by this method. The dyes produced by all phenolic compounds have a maximum absorbance at the same wavelength. The method has been applied to determination of total phenolic compounds in wastewater (106,110) and surface water (111), 2,4-dichlorophenol in water (86), 1-naphthol in aqueous solutions of carbaryl (88), and phenolic fungicides in fabric (112).

The classical method of analysis for phenolic compounds is based on the formation of dibromoindophenol dyes by the condensation of 2,6-dibromoquinone chlorimide with phenolic compounds having an open para position in alkaline solution (113). The relationship between concentration of a phenolic compound and absorbance of the dye is linear over the range 0 to 10 mg/L in aqueous solution or 0 to 100 μg/L in n-butyl alcohol. The time required for dye formation is dependent on the phenolic compounds being determined with up to 24 hours required for complete color development of some compounds. Another inherent problem is that the reaction products of different phenolic compounds in combination with this colorimetric reagent have absorption maxima at different wavelengths (106,114). An advantage of this is that specific phenolic compounds such as resorcinol can be determined in the presence of phenol by measuring the absorbance of the dibromoindophenol dye at 530 nm immediately after the addition of reagents to the sample (115).

A method for determination of phenolic compounds based on the formation of indophenol by the reaction with N-(benzenesulfonyl) quinonimine was reported (116). The reagents used are much more stable than 2,6-dibromoquinone chlorimide, and color formation is complete within three minutes. The concentration of phenolic compounds is proportional to the initial rate of formation of indophenol and to the absorbance of indophenol after three minutes. Unfortunately, the wavelength of maximum absorbance ranges from 610 to 650 nm depending on the specific phenolic compound. Lowest detectable concentrations range from 0.6 to 10.0 mg/L for seven phenols determined.

A promising method for quantification of total phenolic compounds which has been automated (117,118) is based on the oxidative coupling reaction of 3-methyl-2-benzothiazolinone hydrazone with phenolic compounds in an acid solution of ceric ammonium sulfate to from an azo dye. The reaction is relatively insensitive to pH, produces a more intensely colored product than the 4-aminoantipyrine procedure, and occurs with ortho-, meta-, and para-substituted phenolic compounds. Aromatic amines and aliphatic aldehydes react to give a falsely high result if not removed from the sample prior to analysis (117).

Most spectrophotometric absorption methods depend on the reaction of the aromatic ring of phenol with reagents to produce a compound with a high

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molar absorptivity. Ionization of the phenol hydroxyl group when the pH is shifted from acidic to basic is the basis of spectral UV methods. Sensitive methods for quantification of phenolic compounds based on this bathochromatic shift of the long wavelength maximum which occurs when phenols are made basic have been developed for analysis of refinery wastes (91,119), synthetic rubber latex and rubber products (120), and serum (121). A method was proposed for quantifying phenolic compounds by monitoring the absorbance at a wavelength of 292.5 nm (119). This wavelength was selected because it is an intermediate value for the range of absorption maxima exhibited by a group of representative phenolic compounds. P-cresol was selected as the calibration standard because it has an absorption maximum at 292.5 nm which is similar to the absorption maximum of a selected group of phenols. The shift of the wavelength maximum can be quantified by measuring the absorbance before and after pH adjustment with a standard single or double beam ultraviolet spectrophotmeter or with an ultraviolet ratio spectrophotometer specially designed for this technique (122,123). Phenol, rather than p-cresol, is used as the standard with the ultraviolet ratio spectrophotometer. The method is sensitive to all except hindered phenolic compounds which do not ionize fully, such as those having a butyl or larger group in the 2 or 6 position (91). Concentrations of phenolic compounds as low as 0.25 mg/L were determined by differential absorbance measurements using a double beam ultraviolet spectrophotometer with 5 cm absorption cell (118).

Titrimetric Methods--

There are many suitable titrants and solvents (124) for volumetric determination of phenolic compounds. Titrants used in most cases are not specific for phenolic compounds. Examples of these are the oxidimetric titrants N,N'-dibromo-p-toluenesulphonamide (dibromamine-T) (125) and iodobenzene dichloride (126) which are used in acetic acid medium for potentiometric quantification of many diverse reductants and the titrant 1,2,4,6-tetraphenylpyridinium acetate which is used for potentiometric precipitation titration of nitrophenols, dinitrophenols, and some halogenated phenols, as well as nitroform and nitroform yielding compounds (127). Many volumetric determinations are based on the bromination of phenolic compounds (126,128-131). These methods are suitable for quantification of a specific phenolic compound in a solvent containing no other compounds that react with the titrant. Catalytic thermometric titrations (132-135) can be used for quantification of phenolic compounds, but the methods generally determine all weak acids, thus requiring the separation of phenols from the sample matrix prior to titration. Titration methods were not considered for evaluation because they lack sensitivity or require special instrumentation.

Chromatographic Methods--

Chromatography has been used extensively to separate and identify phenolic compounds. Gas chromatography (136-139) and capillary column chromatography (140) with electron capture (141) or flame ionization detectors (90,123,137,142) are used to quantify phenolic compounds. Researchers have prepared chloroacetate (141), O-isobutyloxycarbonyl (142), chlorophenyl-2,4-dinitrophenyl ether (139), and diethyl phosphate (143) deriviatives of phenolic compounds to improve sensitivity. Unlike many of the

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spectrophotometric methods, chromatographic methods are sensititive to all phenolic compounds, but the specificity achieved by chromatography is not required for determination of total phenols. Chromatographic methods are applicable to determination of individual phenolic compounds rather than total phenolic compounds; therefore, they were not considered for evaluation.

Selection of Methods

Methods which appeared to be applicable to the analysis of solid and semisolid environmental samples for total phenolic compounds were selected. The separation technique of choice depends on the matrix being analyzed, the phenolic compounds present, and the expected concentration of the phenolic compounds. The separation technique must be compatible with the determination step which follows. Distillation is a suitable separation method for aqueous samples containing few volatile compounds which could interfere in the determination. The distillation procedure is time consuming but easy to conduct. Extraction and adsorption techniques are useful for concentrating phenolic compounds as well as separating them from the sample matrix. The recovery of a variety of phenolic compounds must be determined to verify the usefulness of the separation technique as part of a method for determination of total phenolic compounds. Solvent extraction and steam distillation were the isolation techniques selected for evaluation.

Selection of procedures for the quantification of phenolic compounds was based on analytical response to the phenolic compounds, accuracy and precision over a wide concentration range, stability of reagents used for the determination, and ease of manipulation. The following methods for determination of total phenolic compounds were evaluated.

1. Spectrophotometric determination based on measurement of the change in absorbance exhibited when the wavelength of maximum absorbance shifts due to a change in pH of the sample solution (119) using the ultraviolet ratio spectrophotometer (122, 123).

2. Colorimetric determination by measurement of the absorbance of the dye produced by the oxidative coupling reaction of 3-methyl-2-benzothiazolinone hydrazone with phenolic compounds (117).

3. Colorimetric determination by measurement of the absorbance of the dye produced by the reaction of 4-aminoantipyrine with phenolic compounds (9).

PHASE II: EVALUATION OF THE METHODS

The purpose of this phase was to eliminate nonviable methods and identify necessary modifications of promising procedures. Development and evaluation of methods was approached as a two-step procedure. Separation and determination of phenolic compounds were treated independently. Two isolation techniques, steam distillation and solvent extraction, were evaluated by application to solid and semisolid environmental samples spiked with

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phenolic compounds. Three determination procedures were tested on samples of distilled water containing phenol.

Three types of solid or semisolid environmental matrices were selected to be test samples from the list of ten of prime interest. The samples were selected to cover the range of characteristics expected in environmental matrices. A pool of fish tissue was prepared by chopping the edible portions of fish into small pieces, homogenizing the tissue in a blender, and freezing it until needed. Lake sediment, consisting of fine and medium sized sand grains, was dried at 103°C and then separated from miscellaneous material such as shells, leaves, and sticks. Domestic sludge was obtained from the drying pond of a sewage treatment plant. The sludge, dried at 103°C, was pulverized with a mortar and pestle. The dried, pulverized sludge was stored at room temperature until used.

Three phenolic compounds--phenol, p-nitrophenol, and 2,4,5-trichlorophenol--were selected for use as model compounds. Selection was based on probability of occurrence in environmental samples, chemical characteristics of the compound, and availability of the ^{14}C-labeled isotopes. The use of radiolabeled compounds permitted evaluation of the efficiency of the separation procedures independently of procedures specific for the determination of phenolic compounds.

Procedures for separation and determination of phenolic compounds were evaluated by performing the procedure on seven replicate samples. Percent recovery, average percent recovery, and standard deviation of the replicates were calculated using the following equations:

$$\text{Percent recovery} = \frac{100\,(O_i)}{T_i}$$

where O_i = observed value

T_i = true value

$$\text{Average percent recovery} = \frac{\sum_{i=1}^{n} P_i}{n}$$

where n = number of samples

P_i = percent recovery of sample i

$$\text{Standard deviation} = \sqrt{\frac{1}{n-1}\left[\sum_{i=1}^{n} P_i^2 - \left(\sum_{i=1}^{n} P_i\right)^2 / n\right]}$$

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Procedures for Isolation

Two types of isolation procedures, solvent extraction and steam distillation, were applied to the separation of phenolic compounds from solid and semisolid environmental samples. These procedures were evaluated by application to samples spiked with radiolabeled phenolic compounds. Samples of a solid matrix were spiked with known concentrations of radiolabeled phenol, *p*-nitrophenol, or 2,4,5-trichlorophenol. Spiking was accomplished by adding a small volume of a solution containing the phenolic compound to the matrix which had been moistened with water to facilitate mixing. For the first several experiments, samples were held for 1 hour after spiking before being treated by a separation procedure. In the majority of experiments, however, samples were allowed to sit for 16 hours to permit adsorption of the phenolic compounds to the matrix.

Controls were prepared with each set of solid samples and consisted of distilled water or other solvents spiked with the same concentrations of radiolabeled model phenolic compounds. The spiking solution and the control samples were subjected to liquid scintillation counting by transferring three replicate 0.5 or 1.0 mL aliquots of the solution to a glass vial containing 10 mL of scintillation cocktail. The counts obtained for the samples from a liquid scintillation counter were corrected for counting efficiency and used to calculate the concentration of phenolic compounds in the sample solutions.

Solvent Extraction--

Two techniques of solvent extraction were investigated. The first technique consisted of stirring the matrix with a solvent using a magnetic stirring bar. Experiments were performed using water at pH 2 as the solvent and also using methylene chloride as the solvent. A 10-g portion of dried sludge was placed in a 250-mL glass bottle, spiked with 2 µg radiolabeled phenol, and allowed to air dry overnight. One hundred milliliters of a solvent was added to the sample in the bottle, and the mixture was stirred with a magnetic stirring bar. Periodically, 5-mL aliquots of the solvent were removed from the bottle and subjected to liquid scintillation counting to determine the recovery of the phenolic compound. The ratio of solvent to sludge decreased with time because of the removal of aliquots for counting. Details of the experiment and percent recovery of phenol are shown in Table 10. Recovery using water acidified with sulfuric acid to pH 2 for the extraction solvent was low as expected. Extraction with methylene chloride yielded a higher recovery. Recovery remained fairly constant over time.

Another set of experiments was conducted using the same procedure, but the volume of solvent removed for liquid scintillation counting, usually 5 mL, was replaced each time with fresh solvent. Recoveries of the phenolic compounds were calculated from liquid scintillation counting data and took into account the dilution effect of adding fresh methylene chloride at each time interval. The average recoveries of phenol, *p*-nitrophenol, and 2,4,5-trichlorophenol extracted from seven replicate samples of fish tissue, sediment, and sludge are listed in Table 11. Recovery tended to be highest after 2 hours of stirring. The average percentage of phenol recovered from

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TABLE 10. AVERAGE PERCENT PHENOL EXTRACTED FROM SLUDGE BY THE STIR-BAR EXTRACTION PROCEDURE WITHOUT VOLUME REPLACEMENT

Solvent	pH of mixture	Extraction time (hour)	Average percent recovery ± standard deviation	Number of replicates
water	2	0	38 ± 6	7
		0.5	41 ± 5	7
		1.0	28 ± 5	7
		2.0	24 ± 8	7
		4.0	20 ± 4	7
methylene chloride	NA*	0	64 ± 9	7
		0.5	79 ± 3	7
		1.0	80 ± 3	7
		2.0	78 ± 3	7
		4.0	78 ± 3	7

* Not adjusted

all three matrices was 87. An average of 85 percent of the *p*-nitrophenol and 80 percent of the 2,4,5-trichlorophenol were extracted from the three matrices.

The second solvent extraction technique evaluated was Soxhlet extraction with methylene chloride as the solvent. A series of experiments was performed to determine the recovery of phenol, *p*-nitrophenol, and 2,4,5-trichlorophenol from sludge, sediment, and fish tissue. Ten-gram portions of each solid matrix were placed in a cellulose extraction thimble and spiked with a solution containing a known concentration (Table 12) of radiolabeled phenol, *p*-nitrophenol, or 2,4,5-trichlorophenol. The spiked samples were allowed to air dry overnight at room temperature. The samples were extracted for two hours with methylene chloride in a Soxhlet extraction apparatus. Controls were prepared by extracting an empty cellulose thimble with methylene chloride containing one of the phenolic compounds to determine losses of phenol to the extraction apparatus. Method blanks were prepared by extracting a cellulose thimble with methylene chloride. Seven replicate extractions of each matrix spiked with each phenolic compound were performed. Aliquots of methylene chloride were analyzed by liquid scintillation counting to determine the amount of radiolabeled phenolic compound extracted. The average recovery and standard deviation of each combination of matrix and phenolic compound are shown in Table 12. The recoveries ranged from 52 to 99 percent and varied with sample matrix and phenolic compound.

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TABLE 11. AVERAGE PERCENT RECOVERY OF PHENOLIC COMPOUNDS EXTRACTED FROM SEVEN REPLICATES OF TEN GRAMS OF EACH MATRIX BY STIRRING WITH METHYLENE CHLORIDE FOR TIMES UP TO FOUR HOURS

	μg of compound added to sample	Average percent recovered ± standard deviation				
		Extraction time (hr)				
		0	0.5	1.0	2.0	4.0
phenol						
tissue	1.3	16 ± 3	72 ± 6	86 ± 9	93 ± 9	94 ± 8
sediment	1.4	66 ± 24	83 ± 16	84 ± 16	84 ± 16	85 ± 13
sludge	1.5	65 ± 5	86 ± 3	86 ± 3	86 ± 3	88 ± 4
p-nitrophenol						
tissue	3.4	14 ± 2	65 ± 4	69 ± 6	73 ± 6	72 ± 4
sediment	3.5	48 ± 8	95 ± 1	96 ± 2	98 ± 3	97 ± 3
sludge	2.0	30 ± 7	72 ± 4	79 ± 6	83 ± 7	88 ± 8
2,4,5-trichlorophenol						
tissue	56.5	11 ± 2	62 ± 4	69 ± 6	72 ± 6	75 ± 4
sediment	31.0	32 ± 5	90 ± 8	94 ± 2	97 ± 2	95 ± 2
sludge	18.0	36 ± 5	68 ± 3	71 ± 4	72 ± 3	75 ± 5

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TABLE 12. RECOVERY OF ^{14}C-LABELED PHENOLIC COMPOUNDS FROM SLUDGE, SEDIMENT, AND FISH BY SOXHLET EXTRACTION WITH METHYLENE CHLORIDE

	^{14}C-Phenol		^{14}C-p-nitrophenol		^{14}C-2,4,5-trichlorophenol	
Matrix	Concentration in sample (µg/g)	Average percent recovery ± standard deviation	Concentration in sample (µg/g)	Average percent recovery ± standard deviation	Concentration in sample (µg/g)	Average percent recovery ± standard deviation
Sludge	0.14	75 ± 7	0.34	73 ± 13	4.6	77 ± 17
Sediment	0.27	90 ± 1	0.67	99 ± 9	2.5	91 ± 10
Fish	0.28	56 ± 5	0.11	52 ± 6	1.6	90 ± 7

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Steam Distillation--

Steam distillation is the conventional procedure for separating phenolic compounds from aqueous samples and was evaluated as a procedure for separating phenolic compounds from solid and semisolid environmental samples. A series of experiments was performed to assess the recovery of phenol, p-nitrophenol, and 2,4,5-trichlorophenol from samples of water and sludge. Ten-gram portions of dried, pulverized domestic organic sludge were prepared for distillation by spiking with a solution of radiolabeled phenol, p-nitrophenol, or 2,4,5-trichlorophenol. The samples were allowed to air dry overnight at room temperature. One hundred milliliters of water was added to the samples, and the pH of the mixture was adjusted to 4 with phosphoric acid. The mixture was gently boiled to distill the water and phenolic compounds. Aliquots of the distillate were counted by liquid scintillation counting periodically to determine the recovery of the phenolic compound. Control samples were prepared by spiking 100 mL of distilled water with a phenolic compound and adjusting the pH to 4 with phosphoric acid. Controls and sludge samples were prepared at the same time and were treated identically. The average percent recoveries of phenol, p-nitrophenol, and 2,4,5-trichlorophenol distilled from controls and sludge samples are shown in Table 13. Recovery of phenol from both the water controls and the sludge samples was 92 to 96 percent. Recovery of p-nitrophenol and 2,4,5-trichlorophenol was less than 5 percent.

TABLE 13. AVERAGE PERCENT RECOVERY OF PHENOL, P-NITROPHENOL, AND 2,4,5-TRICHLOROPHENOL BY DISTILLATION OF AQUEOUS AND SLUDGE SAMPLES

Matrix and compound	µg of compound	Average percent recovered ± standard deviation	Number of replicates
Water			
phenol	402	92 ± 5	7
p-nitrophenol	402	<1	2
2,4,5-trichlorophenol	417	2 ± 1	2
Sludge			
phenol	2	96 ± 7	7
p-nitrophenol	3	<1	3
2,4,5-trichlorophenol	31	<1	5

Procedures for Quantification

Three procedures for the quantification of total phenolic compounds, included in Appendix C, were evaluated by analysis of aqueous solutions of

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phenol. Seven replicate analyses were performed by each procedure on a solution having a concentration approximately ten times that of the lowest calibration standard. One of the procedures evaluated is based on the reaction of phenolic compounds with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10 to form a dye (9). The absorbance of the solution containing the dye is measured at a wavelength of 460 or 510 nm and is proportional to the concentration of phenolic compounds in the sample. The 4-aminoantipyrine reacts with the carbon in the *para*-position of the phenolic compound. The reaction does not take place if the *para*-position is occupied by a substituent other than a carboxyl, halogen, hydroxyl, methoxyl, or sulfonic acid group (144). Materials present in the sample which cause turbidity or color interfere with the determination. The second colorimetric procedure evaluated was based on measurement of the absorbance of a dye produced by the oxidative coupling reaction of 3-methyl-2-benzothiazolinone hydrazone with phenolic compounds. The coupling takes place at the *ortho*-position or at the *para*-position if the *ortho*-position is unavailable (145).

The analytical range, precision, and accuracy of these two procedures, reported in Table 14, are very similar. The reagent 3-methyl-2-benzothiazolinone is able to react with a greater number of substituted phenolic compounds than is 4-aminoantipyrine, and the phenol reaction product has a higher molar absorptivity than the comparable 4-aminoantipyrine product. A disadvantage of the 3-methyl-2-benzothiazolinone procedure is that the compounds produced by reaction with different phenolic compounds have their absorbance maxima at different wavelengths ranging from 460 to 595 nm (145).

The third procedure evaluated for the determination of total phenolic compounds is based on the bathochromatic shift which occurs when the pH of the sample solution is shifted from acidic to basic (119,122,123). The shift in the wavelength of maximum absorbance permits measurement of the phenolic compound concentration using a standard ultraviolet spectrophotometer or an ultraviolet ratio spectrophotometer developed specifically for the purpose by Spectro Products, Inc., New Haven, Connecticut. The detection limit achievable with the Spectro Products instrument is much lower than that obtained using a standard ultraviolet spectrophotometer (Table 14). *P*-cresol was selected as the calibration standard for use with the standard ultraviolet spectrophotometer because it has a wavelength maximum at 292.5 nm, which is similar to the mean wavelength maximum of a selected group of phenolic compounds (119). Phenol was selected for use as the calibration standard for the Spectro Products instrument as recommended by the manufacturer (146).

Selection of Method

The goals of the second phase were to evaluate procedures for isolation and quantification of total phenolic compounds and select the best procedures for further modification and development. The isolation procedure yielding the highest recovery of phenol, *p*-nitrophenol, and 2,4,5-trichlorophenol from samples of fish tissue, sediment, and sludge was solvent extraction performed by stirring the sample with methylene chloride. The recoveries achieved by this procedure ranged from 72 to 98 percent.

TABLE 14. RANGE, PRECISION, AND ACCURACY OF PROCEDURES FOR THE DETERMINATON OF TOTAL PHENOLIC COMPOUNDS

Method	Range (mg/L)	Average ± standard deviation (mg/L)	Average percent recovery
Colorimetric determination by measurement of the absorbance of the dye produced by the oxidative coupling reaction of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with phenolic compounds.	0.004 - 0.060	0.040 ± 0.004	100
Colorimetric determination by measurement of the absorbance of the dye produced by the reaction of 4-aminoantipyrine with phenolic compounds.	0.004 - 0.04	0.040 ± 0.002	108
Spectrophotometric determination based on measurement of the change in absorbance when the wavelength of maximum absorbance shifts due to a change in pH of the sample solution.			
Spectro Products PH-3 instrument using phenol standards			
sample containing phenol	0.002 - 0.10	0.02 ± 0.002	98
Standard ultraviolet spectrophotometer using p-cresol standards			
sample containing p-cresol	1.0 - 30	8.5 ± 0.7	85
sample containing phenol	1.0 - 30	25 ± 1	236

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Phenolic compounds can be extracted from the methylene chloride into a dilute solution of sodium hydroxide and then quantified by one of several procedures. The most sensitive procedure for determination of phenol in distilled water is the one based on measurement of the bathochromic shift of phenolic compounds which occurs when the pH of the solution is changed from 4 to 12 using the ultraviolet ratio spectrophotometer. The procedure is dependent on ionization of the phenol hydroxyl group and independent of the presence and position of substituent groups. Disadvantages of this procedure are that phenolic compounds which exist as tautomers and certain other phenolic compounds such as the highly substituted chlorinated phenolic compounds are not detected because their absorption maxima lie outside of the range for phenol and the phenolate ion. Absorbance can be determined with an ultraviolet spectrophotometer or an ultraviolet ratio spectrophotometer designed specifically for the determination of phenolic compounds. The ultraviolet ratio spectrophotometer is approximately 500 times more sensitive than the standard ultraviolet spectrophotometer. Quantification using an ultraviolet ratio spectrophotometer such as the one manufactured by Spectro Products, Inc., North Haven, Connecticut, was more sensitive for phenol in distilled water and deserves further study.

The 4-aminoantipyrine procedure, however, has been shown to be widely applicable and to have adequate sensitivity for environmental samples. Moreover, results using the 4-aminoantipyrine procedure can be compared to an abundance of accumulated data, where as, phenolic mixtures determined by the 4-aminoantipyrine method and the ultraviolet ratio spectrophotometer would most likely not be linearly related since the substituent effects are not quantitatively related. The ultraviolet ratio spectrophotometer is not as widely available as the standard ultraviolet spectrophotometer in water quality laboratories. For these reasons, the 4-aminoantipyrine method was selected as the quantification method for solid and semisolid environmental samples.

PHASE III: OPTIMIZATION OF THE METHOD

The purpose of this phase was to join the isolation and quantification procedures to produce a method for analysis of solid and semisolid environmental samples. Extraction by stirring with methylene chloride and quantification using the 4-aminoantipyrine procedure were optimized for solid and semisolid samples. Samples of solid matrices such as fish tissue, sediment, and sludge were prepared for extraction by grinding or pulverizing and were spiked with radiolabeled phenol, *p*-nitrophenol, or 2,4,5-trichlorophenol and allowed to air dry for 16 hours. A 10-g portion of sample and 100 mL of methylene chloride were placed in a 250-mL glass bottle, and the mixture was stirred for 2 hours with a magnetic stirring bar. The average percentage of phenol, *p*-nitrophenol, and 2,4,5-trichlorophenol extracted from samples of fish tissue, sediment, and sludge by this procedure as determined by liquid scintillation counting was 84.

A Waring blender was substituted for the magnetic stirring bar in the extraction procedure. The advantage of using a blender instead of a magnetic stirring bar is that sample manipulation is reduced. Frozen samples can be removed from storage, placed directly in the blender to be homogenized and then extracted. Sodium sulfate is added as a drying agent to the frozen sample in the blender, and the mixture is blended to produce a dry powdered sample. The sample is mixed with 100 mL of 0.18M sulfuric acid solution, transferred to a centrifuge tube, and centrifuged to separate the solids from the acid solution. The supernatant acid solution is decanted into a separatory funnel and extracted three times with methylene chloride using volumes of 50, 25, and 25 mL. Centrifugation of the methylene chloride can be used if necessary to break emulsions. Phenolic compounds must be in an aqueous solution to be quantified by the 4-aminoantipyrine procedure. To meet this requirement, the phenolic compounds are extracted from the methylene chloride into 0.005M sodium hydroxide solution. The details of this procedure are presented in Appendix D.

Experiments were performed to determine the optimal conditions for extraction of phenolic compounds from methylene chloride into a dilute sodium hydroxide solution. A methylene chloride extract of sludge was prepared by stirring 50 g of sludge with 1 liter of methylene chloride for 2 hours. The solid particles were filtered from the mixture, and seven replicate 100-mL aliquots of methylene chloride were spiked with one of each of the radiolabeled phenolic compounds to give a concentration of 11 μg/L of phenol, 11 μg/L of p-nitrophenol, or 130 μg/L of 2,4,5-trichlorophenol. Acid-soluble compounds were extracted from the methylene chloride with 15 mL of nitric acid solution and discarded. The phenolic compounds were serially extracted from the methylene chloride with 20, 10, and 10 mL aliquots of 0.005M sodium hydroxide solution. The sodium hydroxide extracts were combined and diluted to 50 mL with 0.005M sodium hydroxide solution. A control consisting of clean methylene chloride was spiked and extracted with each set of sludge-methylene chloride extracts. For all samples, the percentages of the phenolic compounds present in each extract was determined by liquid scintillation counting and are presented in Table 15. The average percentages of phenol, nitrophenol, and trichlorophenol recovered from seven replicates are 74, 89, and 86, respectively. Recovery from the controls was similar, indicating that substances present in the sludge which are soluble in methylene chloride do not reduce the extraction efficiency of the three phenolic compounds. In the final form of the method, the cleanup extraction with nitric acid is not necessary, as the acid-soluble compounds remain in the sulfuric acid-solid mixture and are not extracted into the methylene chloride.

The concentration of total phenolic compounds in the sodium hydroxide solution is determined by the 4-aminoantipyrine procedure. To obtain the clear sodium hydroxide solution required for the 4-aminoantipyrine procedure, it may be necessary to filter the methylene chloride extract of some matrices. Loss of phenol from the methylene chloride by filtration was investigated. Seven replicate solutions of methylene chloride containing 21 μg/L of radiolabeled phenol were filtered through qualitative filter paper. Aliquots of each sample were subjected to liquid scintillation counting before and after filtration. The average recovery of phenol in the seven replicate filtered samples was 95 ± 3 percent.

TABLE 15. AVERAGE PERCENTAGES AND STANDARD DEVIATIONS OF PHENOL, NITROPHENOL, AND TRICHLOROPHENOL EXTRACTED FROM METHYLENE CHLORIDE-SLUDGE EXTRACT

		Sodium hydroxide solution			
Compound	Nitric acid extract	First extraction	Second extraction	Third extraction	total
phenol					
sludge	3 ± 0	45 ± 3	19 ± 1	11 ± 1	74 ± 5
aqueous control	3 ± 0	46 ± 2	16 ± 2	10 ± 1	72 ± 5
p-nitrophenol					
sludge	2	82	3	0.5	89 ± 3
aqueous control	2	83	2	0.3	85
2,4,5-trichlorophenol					
sludge	3	86	2	0.3	86 ± 3
aqueous control	3	86	2	0.3	86

*Number of replicate samples.

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The literature was reviewed for information on compounds which interfere with determination of total phenolic compounds by the 4-aminoantipyrine procedure. Groups of chemicals which are potential interferences are aliphatic aldehydes, reduced sulfur compounds, aromatic amines, and certain metals (108,110). At the pH range of 9.8 to 10.2 at which the reaction is carried out, the tendency of interfering keto-enol systems to respond to the procedure is greatly reduced (108). Most metal and sulfur compounds will not be present in the final methylene chloride extract. Solid and semisolid environmental samples are not expected to contain any interferences which have not been found and investigated in aqueous environmental samples.

The reaction of phenolic compounds with 4-aminoantipyrine in the presence of potassium ferricyanide occurs over a wide pH range. It is important, however, to adjust the pH of the solution to the narrow range of 10 ± 0.2 to insure reproducibility, maximum sensitivity, and minimal response to interferences. The sodium hydroxide solution extract of the methylene chloride has a pH of less than 10. The 4-aminoantipyrine procedure, as recommended by the U.S. EPA (9), specifies the addition of ammonium chloride buffer solution to adjust the pH to 10 ± 0.2. Experiments performed with the sodium hydroxide solution showed that the pH tended to be about 10.5 after addition of the ammonium chloride buffer solution. This problem was remedied by adjusting the pH of the sodium hydroxide solution to 4 by adding a few drops of phosphoric acid solution prior to adding the ammonium chloride buffer solution. This procedure assures a final pH of 10 ± 0.2.

Details of the method for analysis of solid and semisolid environmental samples for total phenolic compounds are presented in Appendix D. Estimates of the precision, accuracy, and sensitivity of the method were calculated from the results of analysis of replicate samples of sludge. Seven replicate 25-g samples of sludge were analyzed for phenol. Three more groups of seven replicate 25-g portions of sludge were spiked with 2, 11, and 100 μg of phenol and analyzed. The direct colorimetric 4-aminoantipyrine procedure was used for quantification of samples containing more than 50 μg of phenol. Chloroform extraction of the colored product was used for measurement of phenol levels less than 50 μg. The micrograms of phenol recovered from each sample are listed in Table 16. The average recoveries of phenol from samples spiked with 2, 11, and 100 μg of phenol were 56, 41, and 45 percent, respectively. The standard deviations of samples containing 0.7, 2.7, 11.7, and 100.7 μg of phenol were 0.3, 0.8, 0.8, and 4, respectively. The coefficients of variation, calculated by dividing the standard deviation by the mean level of phenol recovered, were 43, 53, 17, and 9 percent, respectively, for samples containing 0.7, 2.7, 11.7, and 100.7 μg of phenol. The method is sensitive to approximately 1 μg of phenol. When a 25-g sample is extracted, phenol concentrations as low as 0.04 μg/g can be determined. Further research is needed to improve the recovery of the method.

PHASE IV: VALIDATION OF THE METHOD

The method developed for the analysis of solid and semisolid environmental samples for total phenolic compounds was validated in Phase IV. The

TABLE 16. MICROGRAMS OF PHENOL RECOVERED FROM SPIKED AND UNSPIKED SAMPLES OF SLUDGE

Replicate number	µg of phenol added to sample 0	2	11	100
1	1.3	2.0	4.2	7
2	0.9	0.4	4.0	40
3	0.9	0.6	5.0	50
4	0.6	2.5	6.3	49
5	0.7	1.6	4.8	45
6	0.4	2.3	4.4	42
7	0.4	1.3	4.7	46
average µg recovered	0.7	1.5	4.8	45
standard deviation	0.3	0.8	0.8	4
coefficient of variation	43%	53%	17%	9%
actual µg of phenol in sample	0.7	2.7	11.7	100.7
percent of phenol recovered		56%	41%	45%

method consists of solvent extraction of phenolic compounds with methylene chloride from an acidified slurry of the solid sample. The phenolic compounds are extracted from methylene chloride into dilute sodium hydroxide solution and quantified by the 4-aminoantipyrine colorimetric procedure. The method is presented in Appendix D.

The method for analysis of solid and semisolid environmental samples for total phenolic compounds was validated by analysis of ten sample matrices:

1. lake bottom sediment
2. industrial sludge
3. industrial solid wastes from a disposal site
4. fish tissue
5. microinvertebrates (shrimp)
6. algae
7. soil contaminated by chemicals from the disposal of industrial sludges
8. growing vegetation

9. industrial processing slags disposed of in a landfill
10. commercial incinerator fly ash disposed of in a landfill

One sample of each matrix was obtained, and six 25-g portions of each sample were frozen to preserve the phenolic compounds. Two portions of each sample matrix were removed from the freezer and analyzed to determine the concentration of total phenolic compounds in the samples. The four remaining portions of each matrix were removed from the freezer, thawed, spiked with an aqueous solution of phenol, held for 16 hours to permit adsorption of phenol to the matrix, and analyzed for total phenolic compounds. Two 25-g portions of each matrix were spiked with 20 µg of phenol, and two were spiked with 100 µg of phenol to give concentrations of 0.80 and 4.0 µg/g, respectively.

The concentrations of phenol recovered from the samples are presented in Table 17. The recovery of phenol ranged from 22 to 100 percent with an average of 50 ± 20 percent. Over the concentration range evaluated, recovery was not dependent on concentration of phenol. An average of 50 ± 25 percent of the phenol was recovered from samples spiked with 20 µg, and 50 ± 13 percent was recovered from samples spiked with 100 µg. Recovery does appear to be related to the sample matrix. The average percentage of phenol recovered from the six inorganic matrices--bottom sediment, industrial sludge, industrial solid waste, industrial processing slag, incinerator fly ash, and contaminated soil--was 60 ± 19. An average of 33 ± 8 percent was recovered from the organic matrices which were fish tissue, microinvertebrates, algae, and vegetation.

Quality control samples were extracted and analyzed with each group of environmental samples. One out of every five samples analyzed was a quality control sample consisting of distilled water spiked with phenol. The percentage of phenol recovered from these samples is presented in Table 18. A calibration curve was prepared each day using phenol standards prepared in a 0.005M sodium hydroxide solution. In addition, a reagent-glassware blank was carried through the extraction and quantification procedure daily.

TABLE 17. CONCENTRATIONS OF PHENOL ADDED TO AND RECOVERED FROM SOLID AND SEMISOLID ENVIRONMENTAL SAMPLES

Matrix	Concentration added (μg/g)	Concentration recovered (μg/g)	Percent recovery	Average percent recovery ± standard deviation
bottom sediments	0	ND		75 ± 5
	0	ND		
	0.80	0.64	80	
	0.80	0.62	78	
	4.0	2.8	70	
	4.0	2.9	72	
industrial sludge	0	ND		55 ± 4
	0	ND		
	0.80	0.48	60	
	0.80	0.46	57	
	4.0	2.1	53	
	4.0	2.0	50	
industrial solid waste	0	ND		71 ± 13
	0	0.05		
	0.80	0.56	70	
	0.80	0.72	90	
	4.0	2.5	62	
	4.0	2.5	62	
industrial processing slag	0			81 ± 14
	0			
	0.80	0.80	100	
	0.80	0.68	85	
	4.0	2.9	72	
	4.0	2.7	68	
incinerator fly ash	0	0.20		42 ± 2
	0	0.16		
	0.80	0.52	42	
	0.80	0.48	38	
	4.0	1.9	43	
	4.0	1.9	43	
contaminated soil	0	ND		36 ± 10
	0	0.08		
	0.80	0.24	25	
	0.80	0.28	30	
	4.0	1.8	43	
	4.0	1.8	45	

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Table 17 (Continued).

Matrix	Concentration added (μg/g)	Concentration recovered (μg/g)	Percent recovery	Average percent recovery ± standard deviation
fish tissue	0	0.12		26 ± 3
	0	0.08		
	0.8	0.32	28	
	0.8	0.28	22	
	4.0	1.2	28	
	4.0	1.1	26	
microinvertebrates (shrimp)	0	0.28		39 ± 5
	0	0.32		
	0.80	0.64	38	
	0.80	0.56	32	
	4.0	2.1	44	
	4.0	2.0	42	
algae	0	0.20		34 ± 10
	0	0.12		
	0.80	0.36	25	
	0.80	0.36	25	
	4.0	1.9	43	
	4.0	1.8	42	
vegetation	0	0.16		42 ± 9
	0	0.16		
	0.80	0.40	30	
	0.80	0.48	40	
	4.0	1.9	48	
	4.0	1.9	48	

ND = not detected at concentration above detection limit of 0.05 μg/g

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TABLE 18. RECOVERY OF PHENOL FROM AQUEOUS QUALITY ASSURANCE SAMPLES

Sample	μg of phenol in sample	μg of phenol recovered	Percent
1	20	14	70
2	20	14	70
3	100	80	80
4	100	72	72
5	20	16	80
6	20	14	70
7	100	74	74
8	100	72	72
9	20	14	70
10	20	16	80
11	20	16	80
12	20	16	80
13	100	68	68
14	100	66	66
average ± standard deviation			74 ± 5

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SECTION 5

PRESERVATION OF CYANIDE AND TOTAL PHENOLIC COMPOUNDS IN SOLID AND SEMISOLID ENVIRONMENTAL SAMPLES

INTRODUCTION

The analysis of environmental samples for cyanide and phenolic compounds at trace levels requires methods which are sensitive, accurate, and precise. Therefore, carefully controlled laboratory conditions are essential. To meet these requirements it is generally necessary to ship samples, collected in the field, to a centralized laboratory for analysis. This shipping process can lead to substantial delays between collection and analysis. The effectiveness of sample preservation techniques can substantially affect the accuracy of data generated by the analysis.

The major processes which adversely affect sample integrity are evaporation of volatile compounds, adsorption of more polar compounds on the walls of the sample container, and chemical and/or biological degradation of the sample components. It is possible to slow or eliminate these processes by adding certain chemicals which will not interfere with the analysis to the sample at the time of collection and by refrigerating the sample between the time of collection and analysis (147,148). Unfortunately, a review of the literature revealed a paucity of information on the preservation of samples for analysis for cyanide and phenolic compounds. The few mentions of the employment of preservation techniques for these analyses are restricted to aqueous samples.

Preliminary studies to determine optimal conditions for storage of samples containing cyanide and phenolic compounds were performed. Solid or aqueous samples were spiked with radiolabeled cyanide or phenol, and the concentrations were monitored for 16 days by liquid scintillation counting. Use of radiolabeled compounds allowed losses by volatilization and adsorption to be detected. Loss by degradation of the compounds could not be assessed unless the radiolabeled degradation products were lost from the sample. Three sets of storage conditions were evaluated for preservation of aqueous solutions containing cyanide: storage at less than 0°C, storage at 4-6°C, and addition of sodium hydroxide with storage at 4-6°C. Conditions evaluated for storage of solid samples containing phenol were similar: storage at less than 0°C, storage at 4-6°C, and addition of phosphoric acid and copper sulfate followed by storage at 4-6°C. The most favorable conditions for preservation of samples containing cyanide or phenol appeared to be storage at temperatures of less than 0°C.

Studies were performed to confirm the feasibility of preservation by freezing. Samples of a solid matrix were spiked with cyanide and phenol and

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analyzed by the methods in Appendices B and D. The samples used for validation of the analytical methods for cyanide and phenolic compounds were frozen for preservation.

METHODS OF PRESERVATION

To prevent degradation of sample components during shipment from the field to the laboratory, samples are usually cooled or frozen. Reduced temperatures slow most chemical reactions and decrease biological activity. To eliminate all biological activity, mercuric chloride (149-151) or copper sulfate (9,13) can be added to the sample in the field. It has also been suggested that extremes of pH will halt biological degradation as only a few microorganisms can grow at pH values less than 2 or greater than 10 (152). These techniques can be incorporated into procedures designed to prevent other types of sample decomposition as discussed below.

Cyanide

The most common procedure for preserving samples for cyanide analysis is to add base in the field to raise the pH of the sample to 12 or above (7,9,13). This procedure prevents the formation of the volatile species, hydrocyanic acid. This compound is a weak acid (pK_a of about 10). Conditions of high pH prevent protonation of the cyanide ion and thus keep this species in solution. Oxidizing agents such as chlorine which will decompose cyanide are reduced by adding ascorbic acid prior to pH adjustment (9). Sulfide in the sample will convert cyanide ion to thiocyanate if it is not removed by precipitation with cadmium before preservation. Because cyanides are unstable, analysis within 24 hours of collection is recommended (9,13).

Phenolic Compounds

Generally, samples collected for analysis for total phenolic compounds are acidified in the field and shipped refrigerated to the laboratory (9,13,149). Copper sulfate can be added to the sample at the level of 1 g/L to inhibit biological degradation (9,13,106). Lowering the pH of the sample to approximately 4 insures the complete protonation of the hydroxyl group of even the most polar substituted phenols. Phenol itself has a pK_a of approximately 10, while the pK_a of trichlorophenol is 6. Protonated phenols are less reactive and less polar than the unprotonated form of these compounds. Therefore, the processes of chemical degradation and adsorption onto container walls are slowed or eliminated by acidifying the sample. Nevertheless, it is recommended that samples be analyzed within 24 hours of collection.

EVALUATION OF PRESERVATION METHODS

Samples were spiked with radiolabeled cyanide and phenol to determine optimal preservation conditions. The change in concentration over time was monitored by liquid scintillation counting. The use of radiolabeled cyanide

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and phenol made it possible to detect losses by volatilization and adsorption. Loss by degradation could not be assessed by liquid scintillation counting.

Cyanide

A method for achieving reproducible, high recoveries of cyanide from solid samples had not been developed at the time the preservation study was initiated. For this reason, the preservation conditions were evaluated for samples of water containing cyanide. Semisolid matrices were used for the validation experiments. A slightly basic solution containing 50 µg/L of radiolabeled cyanide as potassium cyanide was prepared. Five-milliliter aliquots of this solution were placed in 15-mL glass vials. The vials were stored under three sets of conditions. One set was stored at 4-6°C and another set at less than 0°C. The third set was treated with 0.5 mL of 2M sodium hydroxide solution to raise the pH to 12 and stored at 4-6°C.

Four samples from each storage group were analyzed by liquid scintillation counting on days 0, 1, 2, 3, 6, 8, 13, and 16. The average percent recoveries of radiolabeled cyanide in aqueous samples under each storage condition are summarized in Table 19. Between day 0 and day 1, an average of 19 percent of the cyanide was lost from all three storage groups. The percentage of cyanide recovered from the samples stored at 4-6°C decreased an additional 14 percent between the first and second days, whereas recovery from the samples stored under the other two conditions decreased only 2 to 3 percent. No further significant loss of cyanide occurred until day 13, when a mean loss of 17 percent was observed in samples stored under all conditions.

TABLE 19. PERCENT RECOVERY OF ^{14}C-LABELED CYANIDE FROM WATER SAMPLES STORED UNDER THREE DIFFERENT CONDITIONS

	Days of storage							
Storage conditions	0	1	2	3	6	8	13	16
<0°C	102	82	80	74	75	75	60	58
4°-6°C	101	80	66	61	54	54	37	29
4°-6°C, NaOH	96	80	77	79	80	82	63	62

Phenol

Preservation studies to determine optimal storage conditions and holding time for solid samples containing phenol were conducted in Phase II us-

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ing samples of sludge and distilled water. Five-gram portions of wet, domestic organic sludge were placed in glass bottles, spiked with 1.8 μg of radiolabeled phenol, and stored under one of three sets of conditions. Five-milliliter aliquots of distilled water in glass bottles were spiked with 0.4 μg of radiolabeled phenol and stored under the same conditions as the sludge samples. One set of water and sludge samples was stored at -7°C, and a second set was stored at 6°C. The third set of samples was treated with 0.5 mL of concentrated phosphoric acid and 0.5 mL of 0.4M cupric sulfate solution and stored at 6°C.

Four samples of sludge from each storage group were extracted with methylene chloride by the stir-bar extraction procedure on days 0, 1, 2, 5, 8, and 16. Radiolabeled phenol in aliquots of the extract was counted to determine the recovery of phenol from the sample. Each day on which sludge samples were extracted, four additional fresh sludge samples were spiked with radiolabeled phenol and extracted. These samples served as controls and permitted monitoring of the extraction efficiency on each day. Aliquots of the water samples were taken directly from the sample bottles and counted. Controls for the water samples were prepared each day by spiking 5 mL of water in a glass bottle with radiolabeled phenol. An aliquot of water from each control was removed for liquid scintillation counting.

The percentages of phenol recovered from samples of sludge and water in each storage group are shown in Table 20. No significant loss occurred from the sludge samples stored at -7°C. The percentage of phenol recovered from the samples stored at 6°C and those treated with acid and copper sulfate and stored at 6°C dropped rapidly to 5 percent by the fifth day. Recovery of phenol from the controls was near 100 percent each day, except day 0 for sludge, indicating that there was no loss of phenol during extraction and handling of samples.

Conclusions

Of the conditions evaluated for preservation of cyanide in water, the most favorable were storage at temperatures of less than 0°C and treatment with sodium hydroxide solution preceding storage at 4-6°C. It is not feasible to mix a solid sample with a solution of sodium hydroxide in the field, although a sample could be immersed in the solution. Samples for cyanide or phenol analysis may be frozen in the field using dry ice.

VALIDATION OF PRESERVATION METHODS

Freezing was tested as a method for preservation of solid and semisolid environmental samples containing cyanide and phenolic compounds.

Cyanide

Samples of sludge from a chemical manufacturing waste treatment process were analyzed for cyanide by the distillation pyridine-barbituric acid method (Appendix B). The samples contained 45 μg/g of cyanide, and therefore, were good candidates for the preservation study. Five-gram portions

TABLE 20. PERCENT RECOVERY OF ^{14}C-LABELED PHENOL FROM SAMPLES OF SLUDGE AND WATER STORED UNDER THREE DIFFERENT CONDITIONS

	Days of storage					
Storage conditions	0	1	2	5	8	16
Sludge						
-7°C	107	100	93	99	98	94
6°C	99	62	16	55	3	3
6°C, H_3PO_4,$CuSO_4$	104	54	17	5	3	2
Control*	84	98	100	103	104	95
Water						
-7°C	103	104	101	97	101	102
6°C	102	104	103	103	103	99
6°C, H_3PO_4, $CuSO_4$	102	103	101	102	101	99
Control*	102	104	103	106	98	103

*Control samples were prepared and extracted each day.

of sludge were placed in glass vials and frozen. Four replicate samples were analyzed for cyanide on days 0, 1, 2, 4, 8 and 16. The concentrations of cyanide measured in the samples are presented in Table 21. No loss occurred over the 16 day period.

TABLE 21. CONCENTRATIONS OF CYANIDE (μg/g) IN SLUDGE SAMPLES STORED AT TEMPERATURES OF LESS THAN 0°C

Replicate number	Days of storage 0	1	2	4	8	16
1	45	51	41	54	38	48
2	50	49	43	56	41	46
3	42	43	37	54	35	47
4	44	42	45	52	38	42
average ± standard deviation	45±3	46±4	42±3	54±2	38±2	46±3

Phenol

A sample of soil contaminated with phenolic compounds was obtained from a chemical manufacturing plant. Replicate 8-g portions of soil were placed in glass vials and frozen. Four replicate samples were extracted and analyzed on days 0, 1, 2, 4, 8, and 16 by the procedure presented in Appendix D. The results of the study, reported in Table 22, show that there was no significant loss of phenol over the 16-day period.

TABLE 22. CONCENTRATIONS OF PHENOL (μg/g) IN SOIL SAMPLES STORED AT TEMPERATURES OF LESS THAN 0°C

Replicate number	Days of storage 0	1	2	4	8	16
1	181	146	164	165	151	175
2	186	159	173	165	160	162
3	191	117*	178	159	177	163
4	149	162	168	143	176	179
average ± standard deviation	177±19	146±20	171±6	158±10	166±13	170±9

* Sample contained a stone. This probably accounts for the lower concentration of phenol.

Conclusions

Storage of samples at temperatures of less than 0°C appears to be an effective means of preserving solid and semisolid environmental samples which are to be analyzed for cyanide or total phenolic compounds. There was no significant loss of cyanide or phenolic compounds from solid, environmental samples stored for 16 days. Advantages of freezing over other preservation techniques are that acids, bases, or other reagents do not have to be taken into the field, sample handling is reduced, and introduction of contaminants is minimized. Freezing was used to preserve the solid and semisolid environmental samples analyzed in the validation study.

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APPENDIX A

PROCEDURES FOR QUANTIFICATION OF CYANIDE

DETERMINATION OF CYANIDE: PYRIDINE-BARBITURIC ACID METHOD (9)

1. Reagents

   1.1 Sodium hydroxide solution, 1.25M: Dissolve 50 g of NaOH in distilled water, and dilute to 1 liter with distilled water.

   1.2 Sodium dihydrogen phosphate, 1M: Dissolve 138 g of $NaH_2PO_4 \cdot H_2O$ in 1 liter of distilled water. Refrigerate this solution.

   1.3 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g of KOH in 1 liter of distilled water. Standardize with 0.0192M $AgNO_3$. Dilute to appropriate volume so that 1 mL = 1 mg CN.

   1.4 Standard cyanide solution A: Dilute 50 mL of stock (1.3) to 1 liter with distilled water (1 mL = 50 µg CN).

   1.5 Standard cyanide solution B: Dilute 100 mL of standard cyanide solution A to 1 liter with distilled water (1 mL = 5 µg CN).

   1.6 Chloramine-T solution: Weigh and dissolve 1.0 g of white, water soluble Chloramine-T in 100 mL of distilled water and refrigerate until ready to use. Prepare fresh weekly.

   1.7 Pyridine-Barbituric acid reagent: Put 15 g of barbituric acid in a 250-mL volumetric flask. Add just enough distilled water to wash the sides of the flask and wet the barbituric acid. Add 75 mL of pyridine and mix. Add 15 mL of concentrated hydrochloric acid (sp. gr. 1.19), mix and cool to room temperature. Dilute to 250 mL with distilled water and mix. This reagent is stable for approximately six months if stored in a cool, dark place.

2. Procedure

   2.1 Prepare a series of standards by pipetting suitable volumes of standard cyanide solution B (1.5) into 250 mL volumetric flasks. To each standard add 50 mL of 1.25M sodium hydroxide solution (1.1) and dilute to 250 mL with distilled water. Prepare as follows:

mL of Standard Solution B (1.0 mL = 5 μg cyanide)	μg of cyanide per 250 mL
0	0
1.0	5
2.0	10
5.0	25
10.0	50
15.0	75
20.0	100

2.2 Withdraw 50 mL of each standard and transfer to 100 mL volumetric flasks. Also withdraw 50 mL of the distilled samples and transfer to 100 mL volumetric flasks.

2.3 Add 15 mL of sodium phosphate solution (1.2) to all of the standards and samples and mix.

2.4 Add 2 mL of Chloramine-T solution (1.6) and mix. Allow to stand 1 to 2 minutes.

2.5 Add 5 mL of pyridine-barbituric acid reagent (1.7) and mix. Dilute to mark with distilled water and mix again.

2.6 Allow 8 minutes for color development, and then read the absorbance at 578 nm in a 1 cm cell within 15 minutes.

3. Calculations

3.1 Prepare a standard calibration curve by plotting absorbance of standard versus cyanide concentration in μg per 250 mL.

3.2 Determine the unknown sample concentration corresponding to the measured absorbance from the calibration curve.

3.3 Calculate the cyanide concentration, in μg/L, in the original sample as follows:

$$\text{Cyanide, } \mu\text{g/L} = \frac{A \times 1{,}000}{B} \times \frac{50}{C}$$

where A = μg cyanide read from standard curve
B = mL of original sample for distillation
C = mL taken for colorimetric analysis

DETERMINATION OF CYANIDE: TRIS (1,10-PHENANTHROLINE)-IRON II COMPLEXATION (46)

1. Reagents

1.1 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g of KOH in 1 liter of distilled water. Standardize with 0.0192M $AgNO_3$. Dilute to appropriate concentration so that 1 mL = 1 mg CN.

1.2 Intermediate cyanide solution: Dilute appropriate volume of the stock cyanide solution to obtain an intermediate working solution having CN concentration of 10.0 µg/mL.

1.3 Disodium hydrogen phosphate solution, 1M: Dissolve 381.37 g of $Na_2HPO_4 \cdot 7H_2O$ and dilute to 1 liter with distilled water.

1.4 Hydroxylamine hydrochloride solution, 10%: Dissolve 100 g of hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) in 1 liter of distilled water.

1.5 Sodium hydroxide solution, 0.5M: Dissolve 2 g of NaOH in distilled water and dilute to 100 mL.

1.6 Thymol blue indicator, 0.1%: Weigh 0.1 g of thymol blue indicator into a 100 mL volumetric flask. Add a small volume of 0.5M NaOH solution (1.5), swirl to dissolve, and dilute to volume with distilled water.

1.7 Ferroin Reagent Solution: Dissolve together 1.96 g of $Fe(NH_4)_2$ - $(SO_4)_2 \cdot 6H_2O$ and 3.17 g of 1,10-phenanthroline monohydrate to a volume of 1 liter using distilled water. The resulting solution is 0.005M ferroin sulfate and 0.001M 1,10-phenanthroline.

1.8 Acetic acid solution, 1M: Dilute 58 mL of glacial acetic acid to 1 liter with distilled water.

1.9 Chloroform

2. Procedure

2.1 Prepare a series of standards by diluting appropriate volumes of the intermediate cyanide solution. Standards should have a CN concentration range of 0.2 to 1.0 µg/mL.

2.2 Measure 25 mL of the standards and the samples into 50 mL glass-stoppered Erlenmeyer flasks.

2.3 Add 5 mL of 1M disodium hydrogen phosphate solution (1.3), 1 mL of 10% hydroxylamine hydrochloride solution (1.4), and 2 drops of thymol blue indicator (1.6). If the indicator does not impart a yellow color to the solution, add 1M acetic acid (1.8) drop-by-drop until the yellow color persists.

2.4 Add 0.5M sodium hydroxide solution (1.5) drop-by-drop until the indicator changes to blue; an intermediate light green color will be observed just before this point is reached.

2.5 Add 5 mL of the ferroin reagent (1.7) and heat in a boiling water bath for 10 to 15 minutes with the glass stopper in place.

2.6 Cool the contents of the flasks and transfer to 60-mL separatory funnels. Extract each solution four times with 5 mL portions of chloroform. Combine the extracts by filtering into 25-mL volumetric flasks and diluting to final volume with chloroform.

2.7 Measure the absorbance of the chloroform extracts at 597 nm in a 1-cm cell using chloroform as the standard blank.

3. Calculations

3.1 Prepare a calibration curve by plotting the absorbance versus concentration of the standards.

3.2 Determine the sample concentration corresponding to the measured absorbance from the calibration curve.

3.3 If the sample was distilled prior to determination, calculate the concentration of cyanide in the original sample as follows:

$$\begin{array}{c}\mu g/mL \\ \text{cyanide in} \\ \text{sample}\end{array} = \frac{A \times B}{C}$$

where A = µg/mL cyanide from curve
B = volume of distillate (mL)
C = volume of original sample taken for distillation (mL)

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DETERMINATION OF CYANIDE: MERCURIC CHLORANILATE - U.V. METHOD (49)

1. Reagents

1.1 Mercuric chloranilate: In a small beaker, gently wash approximately 10 to 15 g of mercuric chloranilate powder 3 or 4 times using small volumes of ethanol. Filter and spread the washed chloranilate in a clean beaker and allow to dry at room temperature.

1.2 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g of KOH in 1 liter of distilled water. Standardize with 0.0192M $AgNO_3$. Dilute to appropriate concentration so that 1 mL = 1 mg CN.

1.3 Intermediate cyanide solution: Dilute appropriate volume of the stock cyanide solution to obtain an intermediate working solution have CN concentration of 10.0 µg/mL.

1.4 Ethanol-water solvent: Mix 300 mL of 95% reagent grade ethanol and 200 mL of distilled water.

1.5 Acid Buffer: Dilute 10 mL of concentrated nitric acid to 100 mL with distilled water.

2. Procedure

2.1 Prepare a series of standards by pipetting appropriate volumes of the intermediate cyanide solution (1.3) into 50 mL volumetric flasks. Dilute to volume with the ethanol-distilled water solvent (1.4). Prepare as follows:

Volume of 10 µg/mL cyanide solution	Concentration of cyanide, µg/mL
0	0
1 mL	0.2
2 mL	0.4
5 mL	1.0
10 mL	2.0
20 mL	4.0
40 mL	6.0

2.2 Prepare samples by transferring an appropriate volume to a 50 mL volumetric flask and diluting with ethanol-water solvent.

2.3 Withdraw 10-mL aliquots from each standard and sample and place in centrifuge tubes. Add 0.01 g of the washed mercuric chloranilate powder (1.1) and cap the tubes. Shake vigorously, allow to stand for 15 to 20 minutes, and then centrifuge at a moderate speed for 20 minutes.

2.4 Adjust the pH of the centrifuged solutions to 4.0 by drop-by-drop

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addition of the acid buffer solution (1.5) and gently shake to mix. Do not attempt to disturb the excess mercuric chloranilate that has settled to the bottom of the tube.

2.5 Read the absorbance of the standards and samples at 330 nm in a 1 cm absorption cell.

3. Calculations

3.1 Plot the absorbance versus the concentration of the standards.

3.2 Determine the sample concentration corresponding to the measured absorbance from the calibration curve.

3.3 If the sample was distilled prior to determination, calculate the concentration of cyanide in the original sample as follows:

$$\begin{array}{c}\mu g/mL \text{ of}\\ \text{cyanide in}\\ \text{sample}\end{array} = \frac{A \times B}{C}$$

where A = µg/mL of cyanide from curve
B = volume of distillate (mL)
C = volume of original sample taken for distillation (mL)

DETERMINATION OF CYANIDE: AMMONIACAL $NiCl_2$ METHOD (5)

1. Reagents

1.1 Stock cyanide solution: Dissolve 2.51 g of KCN and 2.0 g of KOH in 1 liter of distilled water. Standardize with 0.0192M $AgNO_3$. Dilute to appropriate concentration so that 1 mL = 1 mg CN.

1.2 Intermediate working solution: Dilute appropriate volumes of the stock cyanide solution to obtain intermediate working solution having a CN concentration of 10 µg/mL.

1.3 Potassium hydroxide solution, 0.25M: Dissolve 9 g of potassium hydroxide in distilled water and dilute to 1 liter.

1.4 Ammoniacal nickel chloride solution: Dissolve 0.2268 g of nickel chloride in 500 mL of distilled water in a 1-liter volumetric flask. Slowly add 67 mL of concentrated ammonium hydroxide. Wash sides of volumetric flask with distilled water, mix, and dilute to the mark. This solution is 1M in NH_3 and 0.002M in $NiCl_2$.

2. Procedure

2.1 Prepare a series of standards by pipetting suitable volumes of the intermediate cyanide working solution (1.2) into 50 mL volumetric flasks and diluting to volume with the 0.25M KOH solution (1.3).

Prepare as follows:

Volume of 10 µg/mL cyanide solution	Concentration of cyanide, µg/mL
0	0
1 mL	0.2
2 mL	0.4
5 mL	1.0
10 mL	2.0
20 mL	4.0
30 mL	6.0

2.2 Into test tubes or small vials, measure 5 mL of standards and samples.

2.3 Add 5 mL of the ammoniacal nickel chloride solution (1.4) and shake to mix. Let stand for 20 minutes to allow the reaction to occur.

2.4 Read absorbance at 267 nm.

3. Calculations

3.1 Plot absorbance versus concentration of the standards.

3.2 Read the sample absorbance off the calibration curve to obtain the concentration of the sample.

3.3 If the sample was distilled prior to determination, determine the concentration of cyanide in the original sample as follows:

$$\begin{array}{c}\mu g/mL \\ \text{cyanide in} \\ \text{sample}\end{array} = \frac{A \times B}{C}$$

where A = µg/mL cyanide from curve
B = volume of distillate (mL)
C = volume of original sample taken for distillation (mL)

DETERMINATION OF CYANIDE: REACTION OF CYANIDE WITH ORGANIC DISULFIDES USING DIMETHYLFORMAMIDE (51)

1. Reagents

1.1 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g of KOH in 1 liter of distilled water. Standardize with 0.0192M $AgNO_3$. Dilute to appropriate concentration so that 1 mL = 1 mg CN.

1.2 Intermediate cyanide solutions: Make appropriate dilutions from the stock CN solution to obtain intermediate working solutions having CN concentrations of 30 µg/mL and 3.0 µg/mL.

1.3 pH 7 buffer solution: Dissolve 3.549 g of $Na_2HPO_4 \cdot 7H_2O$ and 3.403 g KH_2PO_4 in 1 liter of distilled water. Dilute to the mark.

1.4 pH 9 buffer: Weigh and dissolve 3.8137 g of $Na_2B_4O_7 \cdot 10H_2O$ and dilute to 1 liter with distilled water.

1.5 DTNB solution, 0.001M: Carefully weigh 0.09908 g of 5,5'-dithiobis 2-nitrobenzoic acid. Dissolve in 25 mL of 95% reagent grade ethanol and dilute to 250 mL with the pH 7 buffer solution (1.3).

1.6 DMF, N,N-dimethylformamide: May be obtained commercially.

2. Procedure

2.1 Prepare a series of standards by transferring appropriate volumes of the intermediate cyanide working solutions (1.2) into 50 mL volumetric flasks. Prepare as follows:

Volume (mL)	Intermediate cyanide solution (µg/mL)	Cyanide Concentration (µg/mL)
2	3 ppm	0.12
5	3 ppm	0.3
1	30 ppm	0.6
2	30 ppm	1.2
3	30 ppm	1.8
4	30 ppm	2.4
5	30 ppm	3.0

2.2 Pipette from 0.5 to 5 mL of sample into 50 mL volumetric flasks.

2.3 To the 50 mL volumetric flasks containing the cyanide standards and samples, add 10 mL of pH 9 buffer (1.4), and mix. Add 10 mL of DMF (1.6) and mix. Add 5 mL of DTNB solution (1.5), mix, and dilute to 50 mL with the pH 9 buffer (1.4).

2.4 Read the absorbance at 325 nm within 30 minutes after dilution, using the pH 9 buffer to adjust the spectrophotometer reading to zero.

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3. Calculations

3.1 Prepare a calibration curve by plotting the absorbance versus concentration of the standards.

3.2 Determine the unknown sample concentration corresponding to the measured absorbance from the calibration curve.

3.3 If the original sample was distilled prior to determination, calculate the concentration of cyanide in the original as follows:

$$\begin{array}{c}\mu\text{g/mL of}\\ \text{cyanide in}\\ \text{sample}\end{array} = \frac{A \times B}{B}$$

where A = μg/mL cyanide from curve
B = volume of distillate (mL)
C = volume of original sample taken for distillation (mL)

DETERMINATION OF CYANIDE: ION SELECTIVE ELECTRODE

1. Reagents

1.1 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g of KOH in 1 liter of distilled water. Standardize with 0.0192M $AgNO_3$. Dilute to appropriate concentration so that 1 mL = 1 mg CN.

1.2 Intermediate working solutions: Dilute appropriate volumes of the stock cyanide solution to obtain intermediate working solutions having CN concentrations of 100 µg/mL, 10 µg/mL, and 1.0 µg/mL.

1.3 10M NaOH solution: Dissolve 400 g of NaOH in distilled water and dilute to 1 liter with distilled water. This is the ionic strength adjustor (ISA).

2. Apparatus

2.1 Cyanide ion selective electrode

2.2 Reference electrode

2.3 pH/mv meter

2.4 Magnetic stirrer

2.5 Stir bars

3. Procedure

3.1 Calibration of electrode

3.1a Put 100 mL of distilled water and 1 mL of ISA solution into a 250-mL beaker. Place the electrodes in the solution to a depth of about 3 cm. Begin stirring and set the meter to read millivolts.

3.1b Pipet 1 mL of the stock cyanide solution into the solution. Stir thoroughly. Read electrode potential in millivolts and record.

3.1c Add 10 mL of stock CN solution. Stir thoroughly. Read electrode potential in millivolts and record.

3.1d Determine the difference between the first and second potential readings. Correct electrode operation is indicated by a difference of 58-59 mV, assuming the solution temperature is between 20°C and 25°C.

3.2 Direct measurement

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3.2a Measure 100 mL of the 10 µg/mL cyanide working solution into a 250 mL beaker and add 1 mL of ISA solution. Set the relative mV to 000.0 using the 10 µg/mL standard. Read the electrode potential of the 10 µg/mL solution.

3.2b Rinse electrodes, blot dry, and place in 100 mL of the 1 µg/mL cyanide working solution. Add 1 mL of ISA, stir thoroughly, and read the electrode potential. Record the most stable reading.

3.2c Rinse electrodes, blot dry, and place in 100 mL of the 100 µg/mL cyanide working solution. Add 1 mL of ISA, stir thoroughly, and read the electrode potential.

3.2d Rinse electrodes, blot dry, and place in 100 mL of sample. Add 1 mL ISA. Stir thoroughly. Record the most stable millivolt reading.

3.3 Low level measurement

3.3a Add 100 mL of distilled water and 1 mL of ISA to a beaker. Place electrodes in this solution. Stir thoroughly.

3.3b Add increments of the 10 µg/mL cyanide solution using the table outlined below. Measure the electrode potential after each addition.

Added volume of 10 mg/L cyanide	Resulting concentration mg/L
0.1 mL	0.01
0.1 mL	0.02
0.2 mL	0.04
0.2 mL	0.06
0.4 mL	0.10
2.0 mL	0.29
2.0 mL	0.48

3.3c Rinse electrodes, blot dry, and place in 100 mL of sample. Add 1 mL of ISA solution. Stir thoroughly. Wait for a stable potential reading and record.

4. Calculations

4.1 Direct measurement

4.1a Plot the millivolt readings (linear axis) against concentration (log axis) on semi-logarithmic graph paper.

4.1b Determine the sample concentration from the calibration curve.

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4.1c If the samples have been distilled prior to determination, calculate the cyanide concentration in the original sample as follows:

$$\text{µg/mL of cyanide in sample} = \frac{A \times B}{C}$$

where A = µg/mL cyanide from curve
B = volume of distillate (mL)
C = volume of original sample taken for distillation (mL)

4.2 Low-level measurement

4.2a Plot the millivolt readings (linear axis) against concentration (log axis) on semilogarithmic paper.

4.2b Determine the sample concentration corresponding to the measured potential from the low-level calibration curve.

4.2c If the samples have been distilled prior to determination, calculate the cyanide concentration in the original sample as follows:

$$\text{µg/mL of cyanide in sample} = \frac{A \times B}{C}$$

where A = µg/mL cyanide from curve
B = volume of distillate (mL)
C = volume of original sample taken for distillation (mL)

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APPENDIX B

METHOD FOR ANALYSIS OF SOLID AND SEMISOLID ENVIRONMENTAL SAMPLES FOR CYANIDE

CYANIDE, TOTAL

1. Scope and Application

1.1 This method is applicable to the determination of cyanide in solid and semisolid environmental samples.

1.2 The procedure can be used for determination of cyanide concentrations exceeding 0.5mg/g in a 10-g sample.

2. Summary of Method

2.1 Cyanide as hydrocyanic acid (HCN) is released from cyanide complexes by means of a reflux-distillation operation and absorbed in a sodium hydroxide solution. The cyanide ion in the absorbing solution is determined colorimetrically.

2.2 In the colorimetric determination, cyanogen chloride, formed by reaction of cyanide with chloramine-T at a pH less than 8, reacts with barbituric acid in a solution of pyridine to form a colored product. The absorbance is measured at 578 nm.

3. Definitions

3.1 Cyanide is defined as cyanide ion and complex cyanides converted to hydrocyanic acid by reaction in a reflux system of a mineral acid in the presence of magnesium ion.

4. Sample Handling and Preservation

4.1 The sample should be collected in plastic or glass wide-mouth bottles. Sample bottles should be thoroughly cleansed and rinsed to remove soluble material.

4.2 Samples should be frozen as soon as possible after collection. Samples can be held for up to 16 days without significant loss of cyanide.

5. Interferences

5.1 Interferences are reduced or eliminated by using the distillation procedure. Volatile sulfur-containing compounds are removed during distillation by bubbling the distillate through a lead acetate solution.

6. Apparatus

6.1 Reflux distillation apparatus such as that shown in Figure 1. The boiling flask should be 1 liter in size and equipped with an air inlet tube and condenser. The sulfur-removal vessel should be equipped with an open-ended glass tube, and the cyanide absorber tube should be equipped with a glass tube with fritted end.

6.2 Spectrophotometer suitable for measurement at 578 nm with a 1.0 cm or larger cell.

6.3 Volumetric flasks, 250 ml, 100 ml.

7. Reagents

7.1 Sodium hydroxide solution, 1.8M: Dissolve 72 g of NaOH in distilled water and dilute to 1 liter with distilled water.

7.2 Dilute sodium hydroxide solution, 0.25M: Dilute 140 mL of sodium hydroxide solution (7.1) to 1 liter with distilled water.

7.3 Sulfuric acid, concentrated.

7.4 Magnesium chloride solution: Dissolve 510 g of $MgCl_2 \cdot 6H_2O$ in distilled water and dilute to 1 liter with distilled water.

7.5 Lead acetate solution: Dissolve 30 g of $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ in 950 mL of distilled water. Adjust the pH to 4.5 with acetic acid and dilute to 1 liter with distilled water.

7.6 Sodium dihydrogenphosphate, 1M: Dissolve 138 g of $NaH_2PO_4 \cdot H_2O$ in 1 liter of distilled water. Refrigerate this solution.

7.7 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g of KOH in 1 liter of distilled water. Standardize with 0.0192M $AgNO_3$. Dilute to appropriate volume so that 1 mL = 1 mg CN.

7.8 Standard cyanide solution, intermediate: Dilute 50.0 mL of stock (1 mL = 1 mg CN) to 1000 mL with distilled water (1 mL = 50.0 mg).

7.9 Standard cyanide working solution: Prepare fresh daily by diluting 100 mL of intermediate cyanide solution to 1000 mL with distilled water and store in a glass stoppered bottle. 1 mL = 5.0 mg CN.

7.10 Standard silver nitrate solution, 0.0192M: Prepare by crushing 5 g of $AgNO_3$ crystals and drying to a constant weight at 40°C. Weigh out 3.2647 g of dried $AgNO_3$, dissolve in distilled water, and dilute to 1000 mL (1mL = 1 mg CN).

7.11 Chloramine-T solution: Dissolve 1.0 g of white, water soluble chloramine-T in 100 mL of distilled water and refrigerate until ready to use. Prepare fresh weekly.

7.12 Pyridine-barbituric acid reagent: Place 15 g of barbituric acid in a 250 mL volumetric flask and add just enough distilled water to wash the sides of the flask and wet the barbituric acid. Add 75 mL of pyridine and mix. Add 15 mL of HCl (sp gr 1.19), mix, and cool to room temperature. Dilute to 250 mL with distilled water. This reagent is stable for approximately six months if stored in a cool dark place.

8. Procedure

8.1 Samples consisting of large pieces of solid material such as tissue should be chopped in a blender or ground with a grinder. Place 10 g of wet homogenized sample and 500 mL of distilled water in a 1 liter boiling flask.

8.2 Add 35 mL of sodium hydroxide solution (7.1) to the cyanide absorber tube and dilute if necessary with distilled water to obtain an adequate depth of liquid in the tube. Place 25 mL of lead acetate solution in the sulfur removal vessel. Connect the boiling flask, condenser, absorber, and trap in the train.

8.3 Start a slow flow of air entering the boiling flask by adjusting the vacuum source. Adjust the vacuum so that there is approximately one inch of foam on the surface of the sodium hydroxide solution in the absorber tube. Caution: The air flow rate will not remain constant after the reagents have been added and while heat is being applied to the flask. Adjust the air flow rate as necessary to prevent the solution in the boiling flask from backing up into the air inlet tube.

8.4 Slowly add 25 mL of sulfuric acid (7.3) through the air inlet tube. Rinse the tube with distilled water and allow the air flow to mix the flask contents for 3 minutes. Pour 20 mL of magnesium chloride solution (7.4) into the flask through the air inlet tube and wash down with distilled water.

8.5 Heat the mixture to boiling, taking care to prevent the mixture from backing up into the air inlet tube. Reflux for 2 hours. Turn off the heat but continue the air flow for at least 15 minutes. After the boiling flask has cooled, disconnect the absorber and turn off the vacuum source.

8.6 Drain the solution from the absorber into a 250 mL volumetric flask and bring up to volume with distilled water washings from the absorber tube.

8.7 Prepare a series of standards by pipeting suitable volumes of standard solution into 250 mL volumetric flasks. To each standard add 35 mL of 1.8M sodium hydroxide solution (7.2) and dilute to 250 mL with distilled water. Prepare as follows:

mL of standard solution (1.0 mL = 5 µg CN)	µg of CN per 250 mL
0	0
1.0	5
2.0	10
5.0	25
10.0	50
15.0	75
20.0	100

8.8 Withdraw 50 mL of each standard solution and 50 mL or less of the sample solution from the flask and transfer to 100 mL volumetric flasks. If less than 50 mL of a sample solution is taken, dilute to 50 mL with 0.25M sodium hydroxide solution (7.2). Add 15.0 mL of sodium phosphate solution (7.6) and mix.

8.8.1 Pyridine-Barbituric Acid Method: Add 2 mL of chloramine-T (7.9) and mix. After 1 to 2 minutes, add 5 mL of pyridine-barbituric acid reagent (7.11) and mix. Dilute to mark with distilled water and mix again. Allow 8 minutes for color development, and read absorbance at 578 nm in a 1 cm cell within 15 minutes.

8.8.2 It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and low) be distilled and compared to similar values on the curve to insure that the distillation technique is reliable. If distilled standards do not agree closely with the undistilled standards, the operator should find the cause of the apparent error before proceeding.

8.8.3 Prepare a standard curve by plotting absorbance of standard versus µg of cyanide.

8.8.4 To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard (7.7) or the working standard (7.8) to insure a level of 20 µg/1 or a significant increase in absorbance value. Proceed with the analysis as in Procedure 8.1 using the same flask and system from which the previous sample was just distilled.

9. Calculation

9.1 Calculate the cyanide concentration, in µg/g, in the original sample as follows:

$$CN(\mu g/g) = \frac{A}{B} \times \frac{50}{C}$$

where:
A = µg of CN read from standard curve
B = weight of original sample distilled (g)
C = mL taken for colorimetric analysis

APPENDIX C

PROCEDURES FOR QUANTIFICATION OF PHENOLIC COMPOUNDS

DETERMINATION OF PHENOL: 4-AMINOANTIPYRINE METHOD (9)

1. Reagents

    1.1 Stock phenol solution: Dissolve 1 g of phenol in 500 mL of distilled water and dilute to 1 liter. Add 1 g of $CuSO_4$ and 0.5 mL of H_2SO_4 to preserve.

    1.2 Intermediate phenol solutions: Dilute appropriate volumes of the stock phenol solution to obtain working phenol concentrations of 10 µg/mL and 1 µg/mL.

    1.3 Buffer solution: Dissolve 16.9 g of NH_4Cl in 143 mL of concentrated NH_4OH and dilute to 250 mL with distilled water. Two mL of buffer solution should adjust 100 mL of sample to pH 10.

    1.4 Aminoantipyrine solution: Dissolve 2 g of 4-aminoantipyrine in distilled water and dilute to 100 mL.

    1.5 Potassium ferricyanide solution: Dissolve 8 g of potassium ferricyanide in distilled water and dilute to 100 mL.

    1.6 Chloroform

2. Procedure

    2.1 Prepare a series of standards with phenol concentrations ranging from 0.004 to 0.040 µg/mL using the intermediate phenol solutions (1.2). Total working volume of each standard must be at least 500 mL.

    2.2 To 500 mL of standards and samples in separatory funnels, add 10 mL of buffer solution (1.3) and mix. Adjust the pH to 10 ± 0.2 by adding additional buffer if necessary.

    2.3 Add 3.0 mL of 4-aminoantipyrine solution (1.4) and mix.

    2.4 Add 3.0 mL of potassium ferricyanide solution (1.5) and mix.

    2.5 After 3 minutes, extract with 25 mL of chloroform (1.6). Shake the separatory funnel at least 10 times, let the $CHCl_3$ settle, shake again 10 times, and let the layers separate.

2.6 Filter chloroform extracts through filter paper into 50-mL beakers and cover with watchglasses.

2.7 Read the absorbance of the samples and standards against the blank at 460 nm.

3. Calculations

3.1 Prepare standard curve by plotting the absorbance value of standards versus the corresponding phenol concentrations.

3.2 Obtain the concentration value of the sample directly from the standard curve.

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DETERMINATION OF PHENOL: OXIDATIVE COUPLING REACTION OF 3-METHYL-2-BENZOTHIAZOLINONE HYDRAZONE WITH PHENOLS (9)

1. Reagents

1.1 Stock phenol solution: Dissolve 1 g of phenol in 500 mL of distilled water and dilute to 1 liter. Add 1 g of $CuSO_4$ and 0.5 mL of H_2SO_4 to preserve.

1.2 Intermediate phenol solutions: Dilute appropriate volumes of the stock phenol solution to obtain working phenol concentrations of 10 µg/mL and 1 µg/mL.

1.3 3-methyl-2-benzothiazolinone hydrazone solution, 0.05%: Dissolve 0.1 g of 3-methyl-2-benzothiazolinone hydrazone hydrochloride in 200 mL of distilled water.

1.4 Ceric ammonium sulfate solution: Add 2.0 g of ceric ammonium sulfate and 2.0 mL of concentrated sulfuric acid to 150 mL of distilled water. After the solid has dissolved, dilute to 200 mL with distilled water.

1.5 Buffer solution: Dissolve in the following order: 8.0 g of sodium hydroxide, 2.0 g of EDTA (disodium salt), and 8.0 g of boric acid in 200 mL of distilled water. Dilute to 250 mL with distilled water.

1.6 Working buffer solution: Mix equal volumes of buffer solution (1.5) and ethanol.

1.7 Chloroform

2. Procedure

2.1 Prepare a series of standards with phenol concentrations ranging from 0.004 µg/mL to 0.060 µg/mL, using the intermediate phenol solutions (1.2). Total working volume of each standard must be at least 500 mL.

2.2 To 500 mL of standards and samples in separatory funnels, add 4 mL of 3-methyl-2-benzothiazolinone hydrazone solution (1.3) and mix.

2.3 After 5 minutes, add 2.5 mL of ceric ammonium sulfate solution (1.4) and mix.

2.4 After an additional 5 minutes, add 7 mL of working buffer solution (1.6) and mix.

2.5 After 15 minutes, add 25 mL of chloroform. Shake the separatory funnel at least 20 times. Allow the layers to separate, and pass the chloroform layer through filter paper into 50-mL beakers and cover with watchglasses.

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2.6 Read the absorbance at 490 nm against a reagent blank.

3. Calculations

3.1 Prepare standard curve by plotting absorbance against concentration values.

3.2 Obtain the sample concentration directly from the standard curve.

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DETERMINATION OF PHENOL: ULTRAVIOLET DETERMINATION OF PHENOL UTILIZING THE SPECTRO PRODUCTS MODEL PH-3 PHENOL INSTRUMENT (146)

1. Reagents

   1.1 Stock phenol solution: Dissolve 1 g of phenol in 500 mL of distilled water and dilute to 1 liter. Add 1 g of $CuSO_4$ and 0.5 mL of H_2SO_4 to preserve.

   1.2 Intermediate working phenol solutions: Dilute appropriate volumes of the stock phenol solution to obtain working solutions containing 1.0 µg/mL and 0.5 µg/mL of phenol.

   1.3 Sodium hydroxide solution, 4M: Dissolve 16 g of NaOH in 75 mL of distilled water. Dilute to 100 mL after the solid has dissolved.

   1.4 Phosphoric acid solution, 22%: Dilute 30 mL of 75% phosphoric acid to 100 mL with distilled water.

2. Procedure

   2.1 Set up and calibrate the spectrophotometer according to the operator's manual. Clean the inside of the 10 cm absorption cell by rinsing several times with phenol-free distilled water.

   2.2 Prepare a series of phenol standards in the range 0.002 to 0.10 µg/mL by diluting appropriate volumes of the intermediate working solutions (1.2) with phenol-free water. Include a standard blank. Adjust the pH of the standards to 4.0 by adding phosphoric acid solution (1.4), drop-by-drop.

   2.3 Rinse the 10 cm cell with the standard blank twice, then fill the cell with the blank solution. Place the cell inside the sample compartment.

   2.4 Adjust the coarse Lamp "A" control to a zero (0) reading on the meter. Set the scale expand to the 10x position and adjust the fine Lamp "A" control to obtain a zero (0) reading on the meter. Return scale expand switch to the 1x position. Open the sample compartment and remove the sample cell. Handle the cell in such a way as not to get fingerprints on it.

   2.5 Add approximately 2 drops of sodium hydroxide solution (1.3) to the cell. Replace the cell in the sample compartment, taking care to be sure that the cell is returned to EXACTLY the original position.

   2.6 Observe the meter reading. Set the scale expand switch to 10x and record the reading.

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2.7 Repeat the steps 2.3 to 2.6 for the phenol standards, beginning with the lowest concentration and working up to the higher concentrations.

3. Calculations

3.1 Divide the meter readings obtained for the phenol standards and samples by 10.

3.2 Plot the corrected meter readings (2.1) on semi-logarithmic graph paper against the known concentrations of the phenol standards.

3.3 Obtain the concentration value of the samples directly from the standard curve, using the corrected meter readings (3.1).

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DETERMINATION OF PHENOLS: ULTRAVIOLET DETERMINATION OF TOTAL PHENOLS (119)

1. Reagents

1.1 Stock *p*-cresol solution: Dilute 1.0 g of *p*-cresol to 100 mL with distilled water. 1 mL = 10 mg *p*-cresol.

1.2 Intermediate *p*-cresol solutions: Dilute appropriate volumes of the stock *p*-cresol solution (1.2) to obtain intermediate working *p*-cresol solutions containing 100 µg/mL and 10 µg/mL p-cresol.

1.3 Sodium hydroxide solution, 4M: Dissolve 16.0 g of sodium hydroxide in 500 mL of distilled water. Dilute to 1 liter.

1.4 Phosphoric acid solution, 22%: Dilute 30 mL of 75% phosphoric acid to 100 mL with distilled water.

2. Procedure

2.1 Prepare a series of *p*-cresol standards in the range 1 to 30 µg/mL using the intermediate *p*-cresol solutions (1.2).

2.2 Take a measured portion of the standards and samples and adjust the pH to 4 with phosphoric acid solution. Read the absorbance of the standards and samples at 293 nm on an ultraviolet spectrophotometer.

2.3 Remove the cell from spectrophotometer, adjust the pH of the solution to 12 by adding a few drops of sodium hydroxide solution (1.3). Place the cell in the spectrophotometer and read the absorbance.

3. Calculations

3.1 Subtract the absorbance of samples and standards at pH 4 from the absorbance at pH 12. Prepare a calibration curve by plotting the difference in absorbance versus concentration values of the *p*-cresol standards.

3.2 The concentration of total phenolic compounds in the sample is obtained directly from the calibration curve.

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APPENDIX D

METHOD FOR ANALYSIS OF SOLID AND SEMISOLID ENVIRONMENTAL SAMPLES FOR TOTAL PHENOLIC COMPOUNDS

PHENOLICS, TOTAL RECOVERABLE

1. Scope and Application

1.1 This method is applicable to the analysis of solid and semisolid environmental samples.

1.2 The method is capable of measuring total phenolic compounds at concentrations greater than 50 ng/g using the direct colorimetric method or at the 5 ng/g level by the chloroform extraction procedure.

2. Summary of Method

2.1 Phenolic compounds are extracted from solid and semisolid samples by blending with dilute sulfuric acid solution. Dichloromethane is used to extract the phenols from the acid solution. The phenolic compounds are extracted from dichloromethane into dilute sodium hydroxide solution. The phenolic compounds present in the sodium hydroxide solution are quantified by the 4-aminoantipyrine colorimetric procedure.

3. Comments

3.1 The extraction efficiency and absorption maxima are different for various substituted phenolic compounds. Because it is not possible to duplicate the mixture of phenolic compounds expected in a variety of environmental samples, phenol has been selected as the standard compound for quantification.

4. Sample Handling and Preservation

4.1 Samples should be collected in wide mouth glass bottles which have been cleaned in a solution of chromic acid.

4.2 Samples should be frozen as soon as possible after collection. Frozen samples may be stored for up to 2 weeks without significant loss of phenol.

5. Interferences

5.1 Compounds which absorb at the analytical wavelength may produce a positive interference.

6. Apparatus

6.1 Blender suitable for laboratory applications

6.2 Beakers, 250 mL

6.3 Plastic centrifuge bottles, 250 mL

6.4 Centrifuge

6.5 Separatory funnels, 250 mL and 500 mL

6.6 Flasks, volumetric, 100 mL

6.7 Conical filter funnels

6.8 Glass fiber filter paper

6.9 Spectrophotometer, for use at 460 nm or 510 nm

6.10 pH meter or pH paper with pH range 3.0 to 5.5 and pH range 9.0 to 12.0

7. Reagents

7.1 Sodium sulfate, anhydrous powder

7.2 Sulfuric acid solution, 0.18M: dilute 10 mL of concentrated H_2SO_4 to 1 liter with distilled water

7.3 Dichloromethane

7.4 Sodium hydroxide solution, 0.005M: Dissolve 200 mg of NaOH in distilled water and dilute to 1 liter.

7.5 Stock phenol solution: Dissolve 1.0 g of phenol in freshly boiled and cooled distilled water and dilute to 1 liter. 1 mL = 1 mg phenol.

7.6 Working solution A: Dilute 10 mL of stock phenol solution to 1 liter with distilled water. 1 mL = 10 mg phenol.

7.7 Working solution B: Dilute 10 mL of working solution A to 1 liter with distilled water. 1 mL = 0.1 mg phenol.

7.8 Phosphoric acid solution, 1 + 9: Dilute 5 mL of 85% phosphoric acid to 50 mL with distilled water.

7.9 Buffer solution: Dissolve 16.9 g of NH_4Cl in 143 mL of concentrated NH_4OH and dilute to 250 mL with distilled water. Two mL should adjust 100 mL of sample extract to pH 10.

7.10 Aminoantipyrine solution A: Dissolve 2 g of 4-aminoantipyrine in distilled water and dilute to 100 mL.

7.11 Aminoantipyrine solution B: Dissolve 0.4 g of 4-aminoantipyrine in distilled water and dilute to 100 mL.

7.12 Potassium ferricyanide solution A: Dissolve 8 g of $K_3Fe(CN)_6$ in distilled water and dilute to 100 mL.

7.13 Potassium ferricyanide solution B: Dissolve 1.6 g of $K_3Fe(CN)_6$ in distilled water and dilute to 100 mL.

7.14 Chloroform

8. Procedure

8.1 Acid Extraction

8.1.1 Place 25 to 50 g of frozen sample in the blender. Blend quickly to a powder.

8.1.2 Weigh a portion of sodium sulfate (7.1) equivalent to the weight of the sample amd add to the sample in the blender and mix.

8.1.3 Add 100 mL of sulfuric acid solution (7.2) to the dry mixture. Blend at high speed for 5 minutes.

8.1.4 Transfer the contents of the blender into a plastic centrifuge bottle. Rinse the blender with two 10-mL portions of the sulfuric acid solution (7.2) and transfer the washings to the centrifuge bottle.

8.1.5 Centrifuge the mixture at moderate speed for 10 minutes. Decant the supernatant fluid from the centrifuge bottle to a 500 mL separatory funnel. Do not disturb the residue at the bottom of the centrifuge bottle.

8.2 To the supernatant acid extract in the 500 mL separatory funnel, add 50 mL dichloromethane (7.3) and extract by shaking for 1 minute. Allow the layers to separate and transfer the dichloromethane to a 250-mL separatory funnel. If the dichloromethane extract is turbid, filter through glass fiber filter paper into the 250 mL separatory funnel.

8.3 Extract the aqueous layer two more times using two 25-mL volumes of dichloromethane, shaking for 1 minute, and separating the layers. Combine all the dichloromethane extracts in the 250-mL separatory funnel, filtering if necessary.

8.4 If an emulsion forms during the extraction with dichloromethane, combine the emulsified layers in a centrifuge bottle. Centrifuge at moderate speed for 5 minutes. Separate the layers and transfer the dichloromethane to the separatory funnel, filtering through glass fiber filter paper if necessary.

8.5 Add 30 mL of sodium hydroxide solution (7.4) to the dichloromethane in the separatory funnel. Extract the phenolic compounds from the dichloromethane by shaking for 1 minute. Filter the sodium hydroxide solution through glass fiber filter paper into a 100-mL volumetric flask.

8.6 Extract the dichloromethane two more times using 15 mL of the sodium hydroxide solution (7.4) for each extraction. Filter and combine the sodium hydroxide extracts in the volumetric flask. Dilute to 100 mL with sodium hydroxide solution (7.4).

8.7 If an emulsion forms during the sodium hydroxide extraction, separate the emulsified layers and combine these in a centrifuge bottle. Centrifuge at moderate speed for 5 minutes, separate the layers, and filter the sodium hydroxide solution through glass fiber filter paper into the volumetric flask. Dilute to 100 mL with sodium hydroxide solution (7.4).

8.8 Direct photometric method

8.8.1 Using working solution A (7.6) prepare the following standards in 100 mL volumetric flasks. Use sodium hydroxide solution (7.4) to dilute to volume.

mL working solution A	concentration (μg/l)
0	0
0.5	50
1.0	100
2.0	200
5.0	500
7.0	700
10.0	1000
20.0	2000

8.8.2 Transfer 100 mL of the sodium hydroxide sample extract or an aliquot diluted to 100 mL with sodium hydroxide solution (7.4) into a 250 mL beaker. Transfer the total volume of standards to beakers.

8.8.3 Adjust the pH of samples and standards to 4 by adding phosphoric acid solution (7.8) drop-by-drop.

8.8.4 Add 2.0 mL of buffer solution (7.9) and mix. The pH of the sample and standards should be 10 ± 0.2.

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8.8.5 Add 2 mL of aminoantipyrine solution A (7.10) and mix.

8.8.6 Add 2 mL of potassium ferricyanide solution A (7.13) and mix.

8.8.7 After 15 minutes, read absorbance at 510 nm.

8.9 Chloroform extraction method

8.9.1 Using working solution B (7.7) prepare the following standards in 100 mL volumetric flasks. Dilute to volume with sodium hydroxide solution (7.4).

mL of working solution B	concentration (μg/L)
0	0
5	5.0
10	10.0
20	20.0
30	30.0
40	40.0

8.9.2 Transfer the standards and 100 mL of the sodium hydroxide sample extract into 250-mL separatory funnels.

8.9.3 Adjust the pH to 4 by adding phosphoric acid solution (7.8) drop-by-drop.

8.9.4 Add 2.0 mL of buffer solution (7.9) and mix. The pH of the sample and standards should be 10 ± 0.2.

8.9.5 Add 3.0 mL of aminoantipyrine solution B (7.11) and mix.

8.9.6 Add 3.0 mL of potassium ferricyanide solution B (7.13) and mix.

8.9.7 After 3 minutes, extract with 15 mL of chloroform (7.14). Shake the separatory funnel at least 10 times, let the chloroform settle, shake again 10 times, and let chloroform settle again.

8.9.8 Filter chloroform extracts through glass fiber filter paper. Do not add more chloroform.

8.9.9 Read the absorbance of the samples and standards against a chloroform blank at 460 nm.

9. Calculation

9.1 Prepare a standard curve by plotting the absorbance value of standards versus the corresponding phenol concentrations.

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9.2 Obtain the concentration value of sample directly from the standard curve.

9.3 Calculate the concentration of total phenolic compounds in the solid sample using the following equation:

$$\mu g/g = \frac{A \times 0.1}{B}$$

where A = concentration of sample as read from calibration curve ($\mu g/L$)
B = weight of solid sample used for extraction (g)

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